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AN EVALUATION OF n-ALKANES AS MARKERS TO ESTIMATE DRY MATTER INTAKE, DIET SELECTION AND SOLID DIGESTA PASSAGE RATES IN RUMINANTS

Thesis submitted to The University of Edinburgh
for the degree of Doctor of Philosophy

by

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DECLARATION

I declare that the thesis presented here has been composed by me. The experimental work and analyses were carried out by myself, with the assistance of other people as indicated in the acknowledgements. The work in this thesis has not been submitted for any other degree or qualification.

Andrew M. Magadlela

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This work is dedicated to Songezo and Sinazo.

ABSTRACT

Study I (exp 1, 2, and 3) assessed the use of alkanes for estimating diet composition and intake in lambs. In exp 1, 36 lambs at 30% and 45% of projected mature sizes were used in a factorial design with 2 breeds (Suffolk and Scottish Blackface), two sexes (male and female) and three feed treatments [pelleted lucerne (*Medicago sativa*) or pelleted ryegrass (*Lolium* spp) alone or both as a choice] to compare alkane-derived estimates of dry matter intake (DMI) and selected diet with direct measurements. In exp 2, to assess diurnal variation in n-alkane concentration in the gut, 6 restricted and 6 *ad libitum* fed lambs at 45% mature size were used in a split-plot design to compare the ratios of the amount of dotriacontane (C_{32}) to tritriacontane (C_{33}) in the faeces collected at 4 hourly intervals over a 24 hour period. In exp 3, to evaluate the efficacy of dosing once-daily (cf. twice-daily) with C_{32}, 6 once-daily and 6 twice-daily dosed lambs were used in a split-plot design to compare faecal alkane ratios of C_{33} to C_{32}. Exp 1 suggested general agreement between measured quantities and those estimated using the alkane technique However, at 30% mature size, for the lambs fed on grass only, dry matter intake was slightly overestimated and for those fed on lucerne and grass as a choice, dry matter intake of lucerne was underestimated. Exp 2 indicated that the ratio of the concentration of C_{33}:C_{32} in the faeces was not affected by sampling time and thus no diurnal variation irrespective of whether the lambs were restricted or *ad libitum* fed. Experiment 3 suggested no difference in the ratios of the concentrations of C_{33}:C_{32} between the two dosing strategies until the fifth day of dosing. Study II used the alkane pair of C_{36} and C_{35} to compare the voluntary dry matter intake of *Brachiaria decumbens* by 8 dry

cows, averaging 301 kg liveweight and 8 lactating Criollo cows, averaging 320 kg liveweight during the wet season and 8 lactating cows averaging 300 kg liveweight and 7dry cows, averaging 289 kg liveweight during the dry season of Bolivia. Results suggested that this alkane pair can be used to estimate the dry matter of intake of tropical forages, but if the concentrations of C_{35} are very low the accuracy of the estimate may be compromised. Studies III and IV sought to validate a conceptual model which proposed the involvement of buoyancy and size in the rate of passage of particulate digesta from the rumen. Study III used 4 ruminally cannulated Scotttish Blackface wethers, fed on pelleted grass *ad libitum* and on bailed hay *ad libitum*, in a cross-over design to investigate the rates of passage [expressed as mean retention time in the rumination turnover pool (MRT_1), in rumen escape pool (MRT_2) and in the whole digestive tract (MRT_T)] of n-alkane-coated fibre particles of two sizes (large and small) and two densities (dense and buoyant), in a factorial fashion, in the gastrointestinal tract. The small particle pool had a shorter ($p<0.001$) MRT_1 and MRT_T than the large particle pool. In addition, there was a diet and particle size interaction ($p<0.005$) for MRT_1, suggesting that the difference in MRT_1 between the two particle pools was more pronounced when the sheep were fed on hay. There was no difference in MRT_2 between the large and small particle pools. There was a diet and particle size interaction ($p<0.001$) in MRT_2; large particles were held 5 h longer than small particles when the sheep were fed on hay, compared to 2 h when they were fed on pelleted grass. There was no difference in MRT_1 between the buoyant and non-buoyant particle pools and no dietary influence on the MRT_1 of the particles. There was a particle size and buoyancy interaction ($p<0.05$) such that large particles of the dense fraction had a 5 h longer MRT_1 than

smaller ones whereas large particles of the buoyant pool were retained 1.5 h longer than small ones. The non-buoyant pool remained in the tract 4.7 h longer ($p<0.001$) than the buoyant pool. Study IV used four sizes of a tropical cattle breed, Criollo and one size of a mature Criollo X Holstein cross in a split-plot design to compare the rates of passage of the same fibre particles used in Study III in the digestive tracts of cattle. Across all cattle groups the large particles had longer MRT_1 than small particles. For all but one group MRT_2 for small particles were shorter than those for large particles. For MRT_T, the large particles were retained for over 8.5 h longer ($p<0.001$) than the small particles. There was no effect of buoyancy on MRT_1 and MRT_2, but non-buoyant particles displayed a 2 h longer ($p<0.05$) MRT_T than the buoyant ones. There was no apparent effect of animal size or genotype. The 4 studies showed that n-alkanes could be successfully used to estimate DMI, diet selection and solid digesta passage rates in sheep and cattle. Studies III and IV suggested the involvement of buoyancy and size in particulate digesta retention in or passage out of the rumen, but full support for the proposed model could not be justifiably claimed.

CHAPTER 1

Introduction

Grazing animals can vary the composition and thus the quality of their diet by selecting certain plant parts and plant species that may differ substantially in nutritional value, making the composition of selected herbage as important as the amount consumed when quantitatively evaluating the nutritional status of grazing animals. However, both dry matter intake and diet selection are difficult to measure for animals at pasture. The cuticular wax of plants contain many compounds including various alcohols, diketones and hydrocarbons, which in recent years with the improvement of chromatographic separation techniques, have been finding more use in taxonomic studies (Dove and Mayes, 1991). Of particular importance, especially in nutritional studies, have been the saturated hydrocarbons, alkanes because of their widespread nature among plants and their relative ease to analyse by gas chromatography. In most plant species long chain alkanes, ranging from pentacosane (C_{25}) to pentatriacontane (C_{35}), are present with the odd-chain ones predominating. The alkane profiles of plants usually vary from species to species and even from one plant part to another. There may also be within species differences in alkane content that are due to stage of maturity, cultivar, environment, seed origin and plant individuality, but these differences are usually smaller than between species differences (Dove and Mayes, 1991). The differences in the alkane profiles of different species have been exploited to resolve herbage mixtures into proportions of component species (Dove, 1992).

The most commonly used method of estimating dry matter intake, which uses chromium oxide as a faecal output marker and in vitro digestibility estimates does not take into account animal individuality (Chen et al., 1999; Gedir and Hudson, 2000). Digestibility occurring in the animals whose intake is being estimated can be different from the in vitro estimates due to differences in the intake levels of the test animals and the animals used to derive the in vitro calibrations (Dove et al., 2000). Therefore estimates derived by using this method inherently have a certain degree of error about them that does not fully address individual differences, which can be due to differences in physiological status of the animals.

Estimation of intake from quantifying ingestive behavioural attributes like bite rate, bite weight and grazing time may provide good herbage intake data at the time of observation, but it often does not give accurate intake information outside the times during which the behavioural characteristics were observed (Moore and Sollenberger, 1997). Other methods like the estimation of intake from herbage mass disappearance are even more erroneous and labour intensive and are more applicable to intake estimates of groups of animals (Moore and Sollenberger, 1997).

Likewise most methods to estimate the composition of the diet selected by grazing animals, particularly after chewing, are either laborious or yield unsatisfactory estimates. For instance using microscopy for separating oesophageal fistula extrusa into component species is tedious and difficult (Dove and Mayes, 1991). Pinitol, because it is found in legumes and not in grasses, has been used as a chemical marker to estimate the legume content of herbage consumed by steers grazing a grass and legume sward and yielded results which underestimated the proportion of legumes in the diet because some of it seemed to leach out by saliva (Forwood et al., 1987).

Although alkanes have found uses in other areas of nutritional studies like intake estimation and rates of passage determination, their most valuable application is in the estimation the botanical composition of the diet of individual grazing animals because of the inadequacy of other methods (Dove and Mayes, 1991). Unlike the collection and separation of oesophageal fistula extrusa, which provides botanical composition data about the fistulated animal (but not necessarily other animals) over a short period, the alkane method, gives the botanical composition of each animal over a long period of time. The method determines diet composition by matching the patterns of alkane concentrations in the faeces, after correcting for losses in the gut, with those of individual plant species of the diet (Dove and Mayes, 1996).

Intake estimation involves dosing animals with a known amount of an even-chain alkane that is similar in recovery rate to another odd-chain alkane naturally found in the forage (McMeniman, 1997; Dove and Mayes, 1996). If dry matter intake and diet selection are both investigated in the same study, intake determination requires no additional analyses to that done for diet selection, except the dosing of the even-chain alkane and establishment of the amount of the even-chain that is delivered daily. However, cyclic variations of dosed even-chain n-alkanes, which could compromise the accuracy of the technique have been reported (Dove and Mayes., 1991, Dillon and Stakelum, 1990). Also, the commonly used method of administering alkanes to animals is by dosing twice daily with either paper pellets or gelatin capsules, yet dosing once daily would have the advantage of reduced animal handling, but can cause variation with time in the concentration of the dosed alkane (Dove and Mayes, 1991).

Alkanes can also be used as markers to investigate the passage of solid digesta down the gastrointestinal tract of ruminants (Dove and Mayes, 1991). It would appear as if the natural alkanes because of their association with solid digesta would be the most appropriate, but the means of dosing and monitoring would be difficult because they are naturally part of the herbage (Dove and Mayes, 1991). Radioactive labelling is an option (Dove and Mayes, 1991), but health risks and access to analytical equipment would be disadvantageous. Even-chain alkanes can be easily attached to forages and their affinity for solid digesta shows a potential for their use as markers for investigating the rate of passage of solid material through the gut (Mayes et al. unpublished data). Another advantage of using alkanes as markers is that several types are available and therefore several particle pools can be marked, dosed and their passage rates monitored at the same time. However, it is not known whether addition of n-alkanes to forage particles would interfere with the degradation of the forage to which they were attached when used in moderate amounts.

The broad objective of this research was to evaluate the use of alkanes as markers in intake, diet selection and solid digesta passage rates. The research was divided into two intake research initiatives, studies I and II and two rates of passage research initiatives, studies III and IV. The objectives of study I, which comprise three experiments, were to evaluate the feasibility of alkanes as markers for estimating dry matter intake and diet selection by ruminants given diet mixtures (experiment 1), to assess the consequences of diurnal variation in alkane concentration in the gut on the accuracy of the n-alkane technique in estimating dry matter intake (experiment 2) and to test the efficacy of dosing once daily, as opposed to twice daily, with an even-chain alkane (experiment 3). Study II used the alkane pair of C_{36} and C_{35} to compare the voluntary dry matter intake of *Brachiaria decumbens* by dry and lactating cows during the wet season and dry and lactating cows during the dry season of Bolivia.

Studies III and IV sought to validate a conceptual model which proposed the involvement of buoyancy and size in the rate of passage of particulate digesta from the rumen. To that end study III was conducted to investigate (i) the effects of addition of n-alkanes on fermentation characteristics of forage particles, and (ii) the rates of passage of n-alkane-coated fibre particles of two sizes and two densities in the gastrointestinal tract of sheep. Study IV was conducted to compare 1) the passage rates of fibre particles of two sizes and two densities in the gastrointestinal tracts of four sizes of a tropical cattle breed, Criollo and the rates of passage of the same fibre particles in the mature tropical breed against a similarly sized Criollo X Holstein cross and 2) the voluntary dry matter intake and gutfill in the afore-mentioned cattle sizes and genotypes.

CHAPTER 2

Literature review

Intake and particulate rates of passage

The amount of feed consumed by an animal is one of the most important variables which influence its performance. Consequently, much research effort has been devoted to improving understanding of the mechanisms of intake modulation in order to more accurately predict it. Accurate determination and prediction of intake are useful for the formulation of rations to achieve specific animal responses (Burns et al., 1994). Determination of dry matter intake integrates factors such as plant chemical and physical properties and animal physiological processes. However, the mechanisms by which these plant and animal factors inter-relate to regulate intake are complex and have not been clearly delineated (Forbes, 1995). Several physiological factors have been suggested to control voluntary dry matter intake and these include hypothalamic temperature, blood glucose concentration, body fat stores and plasma amino acid levels (Forbes, 1995; 1986). Leng et al. (1993) stated the importance of the balance of volatile fatty acids and amino acid requirements relative to total volatile fatty acids energy available in intake regulation. Physical distension in the gastrointestinal tract, especially the reticulorumen, due to fill has also been postulated to limit intake, but the physiological status of the animal may affect its fill capacity (Allen, 1996; Forbes, 1995).

Under most modern feeding practices the intake of high quality forages and concentrate feeds is generally high enough to meet most production requirements

(Burns, 1994); in such cases intake is more likely to be limited by physiological factors than by stomach distension (Illius and Jessop, 1996). The amounts of poor quality forages eaten by ruminants even under *ad libitum* feeding conditions are often insufficient to support the level of production of which animals are capable (Forbes, 1993). This low intake may be a result of restricted flow of forage digesta through the gastrointestinal tract, which may cause distension of one or more segments of the gut (Allen, 1996). In such circumstances the amount of dry matter consumed by a ruminant during a meal is influenced by the rate of clearance from the rumen of previously ingested food (Forbes, 1986). Rumen clearance occurs by solubilization of soluble material, degradation of degradable material and passage of the less digestible residues from the reticulorumen into the omasum (Van Soest, 1988; Kennedy and Doyle, 1993). The rate of degradation of plant cell wall by rumen microbes is influenced by the supply of nutrients from ingested food or from endogenous recycling into the rumen (Wilson and Kennedy, 1996). The rate is depressed if the supply of nutrients is suboptimal for microbial requirements. Microbial activity is also lowered by a low ratio of external surface area per volume of feed particles, which impedes their access to cell wall carbohydrates before colonization and subsequent degradation (Wilson and Kennedy, 1996).

The rate of passage of particles from the rumen depends on the efficiency of their comminution into small particles, mostly by ruminative chewing, which is influenced by the physical properties of the particles (Murphy and Kennedy, 1993). The physical characteristics of forage digesta then determine not only the efficiency at which they are digested, but also the speed at which undigested residues pass along the gut. Thus a clear elucidation of the kinetics of digestion of forages of different physical and chemical characteristics in the digestive tract, and the physiological factors that modulate them will benefit efforts to predict and enhance the intake of forages by animals.

Digesta particle size

Several investigations have revealed that digesta particles have to be reduced to a small size to increase not only their probability of escape out of the reticulorumen, but also their rate of passage to the post-ruminal tract (King and Moore, 1957; Poppi et al., 1980). For instance, Ewing and Smith (1917) investigated passage rates of food particles in a steer fed on mixed rations of different cottonseed meal, silage and starch proportions and, after slaughtering the steer, observed that larger or coarser food particles required longer time for passage through the gut of the animal than finer particles. Also, Welch (1967) found that when indigestible polypropylene fibres 7 cm long were placed in the rumen of hay-fed sheep they were recovered in the faeces finely ground whereas 90 % of 30 cm long fibres remained unchanged in the rumen after 28 days. In a study to compare digesta particle size distribution in the reticulorumen, omasum and abomasum of sheep fed two alfalfa hay varieties and four grass hays, Troelsen and Campbell (1968) found a higher proportion of coarse particles in the reticulorumen than in the other compartments.

Such findings suggested that digesta particles have to be broken down to some threshold size of fineness before passing from the reticulorumen and led to the concept of a critical size suggested by Poppi et al. (1980). It divides digesta particles in the rumen into small particles which can escape from the rumen and large particles which cannot. This size has been proposed to be approximately 1 mm for both sheep and cattle (Evans et al., 1973; Poppi et al., 1980), but Kennedy and Poppi (1984) later suggested a limiting size of 3-4 mm for cattle. Van Soest et al. (1988), arguing against the idea of a constant limiting size, stated that a higher limit was observed when pelleted feeds were given and when intake was increased. In addition, the proportion of large particles in the reticulorumen of cows fed at several levels of restricted intake was found to increase with higher levels of intake (Okine and Mathison, 1991a). However, Faichney (1993), in agreement with the 1 mm and 3 mm critical size proposals for sheep and cattle respectively, argued that the critical size of particles for passage from the rumen is relatively unaffected by grinding and pelleting feed or by level of feed intake. The suggestion that the critical size increases when intake increases was based on observed increase in faecal mean particle size, an observation which offers limited information on critical size (Faichney, 1993). Although there maybe disagreement over the constancy of the limiting size, reducing digesta particle size does not only speed their passage rate, but it also increases their rate of fermentation in the digestive tract as a result of an increase in surface to mass ratio for microbial attack (Gerson et al., 1988). Furthermore, in their review of physical and chemical variables influencing particle passage, Ehle and Stern (1984) stated that larger particles promote slower passage and do not allow as great a nutrient intake as smaller particles.

Particle size discrimination

Because the average size of digesta particles in the rumen was observed to be larger than that of digesta taken from post-ruminal sites (Poppi et al., 1980), the reticulo-omasal orifice was proposed to be the part responsible for prevention of passage of particles larger than the critical size (Welch, 1982). Welch and Smith (1978), not convinced of the proposed passage limiting role of the reticulo-omasal orifice, carried out an experiment, using polypropylene ribbons of varying lengths, to determine the particle size necessary for passage from the rumen of sheep and cattle without additional breakdown by rumination. They observed that polypropylene ribbons 2 cm long passed through the reticulo-omasal orifice of cattle fed 2 kg of concentrate per day and hay *ad libitum,* albeit in smaller quantities than 1.5, 1.0 and 0.5 cm lengths. Similarly, ribbons 1 cm in length passed through the reticulo-omasal orifice of sheep, but in smaller amounts than shorter lengths (0.25 and 0.5 cm). Furthermore, in an endoscopic study of digesta transfer from the reticulorumen to the omasum of a cow fed long alfalfa (*Medicago sativa*) hay, McBride et al. (1984) observed particles as large as 10 mm to freely go through the reticulo-omasal orifice, but were seldom in a rumen location which predisposes them to delivery to the orifice. They therefore postulated that the screening process of digesta for passage out of the rumen took place somewhere else in the reticulorumen, not at the orifice, although the omasum may return large particles to the reticulum or reduce their size before onward passage.

Evans et al. (1973), after observing changes in the physical characteristics of the digesta in the reticulorumen of cows fed pasture hay once daily, reported that small and dense particles are found in highest concentrations in the cranial and ventral sacs of the rumen. Sutherland (1988) used sheep fed chaffed lucerne (*Medicago sativa*) hay to examine how the forestomachs of ruminants achieves preferential retention of large particles while small particles pass to the postruminal tract. He confirmed the observation made by Evans et al. (1973) that particles in the ventral region of the rumen were generally small and enjoyed preferential passage through the reticulo-omasal orifice and proposed that there is a floating raft in the dorsal rumen, which functions as a discriminating mechanism with high selectivity for large particles. It therefore follows that the particulate digesta which are presented to the orifice for passage must first be processed into small particles and occupy a ventral location in the rumen, since it is particles from this region that are pushed through the orifice. However, there is a possibility of another sorting mechanism in the omasum where large particles are selectively retained and returned to the reticulum as was observed by McBride et al. (1984) in the cow fed long alfalfa (*Medicago sativa*) hay and by Waghorn et al. (1986) in sheep fed chaffed lucerne (*Medicago sativa*).

Particle comminution

Large particle breakdown involves interactions among feeds, microbes and the host animal, but, according to McLeod and Minson (1988), it largely occurs by ingestive and ruminative chewing, with microbial action and detrition contributing insignificantly. However, Wilson and Kennedy (1996) in their review of plant and animal characteristics associated with fibre breakdown argued that, depending on forage species, premaceration digestion in the rumen may increase the fragility of some plants, with plant parts affected differently. Also, plant characteristics and form of presentation exert an influence on the ease, extent and pattern of breakdown of particles by comminution processes (Wilson and Kennedy, 1996). The influence of forage type on chewing time was studied by Minson (1990) who wrote that it took sheep longer to chew tropical than temperate grasses. Chewing time increases with forage maturity (Weston, 1985) and generally leaf fractions are chewed less than stem fractions (McLeod and Smith, 1989), but ruminative chewing is normally about 8 or 9 h per day and increasing the load of indigestible fibre does not increase rumination appreciably beyond that duration (Welch, 1982). Chopping forages to a short length generally reduces chewing time (Weston, 1985; Kennedy, 1995). Highlighting the importance of plant characteristics in particle kinetics, Weston and Kennedy (1984) concluded that even though the probability of passage of particles from the reticulo-rumen is inversely proportional to their size, the passage rates of particles of comparable size but from different plants may vary. Particle comminution through chewing and rumination affects passage rates from the rumen not only by reducing particles to an optimum size for passage, but also by increasing particulate specific gravity due to increased rate of hydration (Hooper and Welch, 1985).

Specific gravity

Although digesta particles have to be reduced in size to be able to flow out of the reticulorumen into the post-ruminal tract, particle reduction does not guarantee passage through the reticulo-omasal orifice. Welch (1982) reported that more than half the dry matter in the rumen of experimental cows could pass a 600 micrometer sieve, but could not be cleared from the rumen of the cows. Data from several other experiments (Evans et al., 1973; Poppi et al., 1981) suggested that most of the particulate matter in the reticulorumen was smaller than the reticulo-omasal orifice and that the reticulo-omasal orifice seemed large enough to accommodate particles larger than the ones found in the faeces of ruminants (Kennedy and Doyle, 1993; McBride et al., 1984). Thus, there is another characteristic by which particles are sorted in the reticulorumen for onward flow.

Specific gravity appeared to be another physical characteristic that determines the behaviour of particles in the digestive tract when King and Moore (1957) observed that plastic particles of density 1.2 g/ml had the most rapid passage rate through the gastrointestinal tracts of cows fed about 2 kg of grain daily and mixed hay *ad libitum*. Data presented by desBordes and Welch (1984) also suggested that plastic particles of specific gravity 1.17 passed fastest through the digestive tract of cows fed mixed grass hay twice daily. Murphy et al. (1989) used a four-pool model study incorporating passage of ruminated and nonruminated particles from the reticulo-rumen, post-ruminal passage, rate of chewing and lag times to compare passage and rumination of inert particles varying in length and specific gravity. Particles of specific gravity of 1.34 had the fastest passage rates from the reticulorumen of swamp buffaloes and cattle given a diet predominantly of rice (*Oryza sativa*) straw *ad libitum*. Kaske and Engelhardt (1990) detected that plastic particles with densities 1.22-1.44 g/ml had the shortest retention time in the gastrointestinal tract of sheep maintained on a diet of medium quality hay given *ad libitum*. However, a recent study (Kennedy, 1995) could not establish a connection between the specific gravity of particles, measured as particle sedimentation rate (Sutherland, 1988), in the reticulum and their probability of passage to the post-ruminal tract of swamp buffaloes and cattle fed mainly on rice straw.

Rumen stratification

It has been found that specific gravity of rumen digesta varies according to rumen site and time of sampling after eating; for instance, Evans et al. (1973) found that soon after feeding the dorsal contained larger and lighter particles than the ventral rumen of nonlactating Friesland cows fed pasture hay. Sutherland (1988) observed no significant difference in particle size or density between the contents of the anterior and posterior rumen of sheep. However, the dorsal rumen was found to be occupied by a floating raft of large and buoyant particles while the ventral rumen contained small and dense particles. Initially upon ingestion forages, except grain, are coarse and buoyant because of air spaces within their tissue structure, but this buoyancy may be lost with time spent in the rumen owing to hydration and the buoyancy will again be temporarily restored after microbial colonization and digestion because of the production of microbial fermentation gases (Kennedy and Doyle, 1993). These findings lent support to earlier proposals that rumen digesta are stratified according to particle size and buoyancy (Evans, 1973; Faichney, 1986). This stratification is most pronounced just after feeding as newly-ingested digesta have not been fully hydrated and comminuted by rumination and digestion (Wilson and Kennedy, 1996), processes which increase particle density (Hooper and Welch, 1985).

The raft in the rumen is kept afloat by fermentation gases which are released as a result of microbial attack within the plant material (Sutherland, 1988). It may entangle some large particles, especially those of rough-surfaced tropical grasses (Wilson et al., 1989) and some small particles that would otherwise pass freely to the post-ruminal tract (Faichney, 1986). Welch (1982), having found no evidence of particle entrapment in ingesta mass limiting presentation of small particles to the reticulo-omasal orifice, investigated other mechanisms that may be involved in particle discrimination. He found that 0.5 cm long flat ribbons passed more rapidly and the recovery of their nonruminated fraction greater than that of cylinders of the same length, which suggested the possible involvement of shape in particle discrimination mechanisms.

The raft has been postulated to be actively involved in one of two mechanisms sorting rumen particles for onward passage to the post-ruminal tract (Sutherland, 1988). Both mechanisms work on the difference in buoyancy between large and small particles. The first, which involves the dorsal raft, supposes that large particles, because of their buoyancy, tend to form a floating raft. Small particles, because of their tendency to sediment, are propelled into the reticulum with the more fluid ventral rumen contents. The second sorting mechanism, which is said to take place in the reticulum, selectively filters large buoyant particles (which may have escaped rumen sorting) for retention in the reticulum and delivers small sedimenting particles to the reticulo-omasal orifice. However, a more recent study by Kennedy (1995) could not detect an obvious dorsal raft of large and buoyant particulate matter in the rumen of cattle and buffaloes; instead large particles from the dorsal rumen sedimented faster than those from the ventral rumen.

Rumination

Specific gravity seems to play a role in the selection of digesta particles for rumination. desBordes and Welch (1984) suggested that plastic particles of specific gravity 1.17 were not only preferentially ruminated, but were also eliminated fastest from the rumen of steers because after rumination they were deposited to a site which predisposes them to quick passage. Murphy et al. (1989) argued that maximum rumination rates occurred at a lower specific gravity than did maximum passage rate of plastic particles through the digestive tracts of buffaloes. The average particle size of digesta decreases but specific gravity increases with time, due to rumination and digestion (Evans et al., 1973). In some instances, however, passage of small particles resulting from ruminative and digestive breakdown may be slower than that of the larger particles from which they originated (Van Soest et al., 1988;

Smith et al., 1983), which suggests specific gravity as a passage limiting factor. Wilson and Kennedy (1996) argue that, because of evidence of entrapment of particles within the dorsal raft, it is unclear whether passage rate is limited more by the time it takes to comminute particles or by the time it takes small particles from the rumen. Such findings serve as a reminder that particle comminution is not the only physical requirement for unhindered passage from the rumen even though particles have to be reduced to go through the reticulo-omasal orifice.

Dietary factors

Rate of passage and thus digestibility is affected by dietary factors. Dietary factors include intake level, type and physical form (Faichney, 1993).

Passage rate and digestibility

Rate of passage of food influences the digestibility of that food; a fast rate lowers digestibility (Weston and Kennedy, 1984). Varga and Hoover (1983) tested *in situ* rate and extent of NDF degradation of two diets of similar chemical composition but different in fill (low and high). The rate and extent of NDF degraded in 24 hours was 0.024 per hour and 43% versus 0.074 per hour and 58% for the high and low fill diets. Shriver et al. (1986) used a continuous culture *in vitro* digestibility system to compare neutral detergent fibre and fat-free organic matter digestibility of a diet of 35% hay and 65% concentrate held for varying periods (to mimic different solid retention times). Their results led them to conclude that the rate of flow of digesta may exert a significant negative effect on nutrient digestibility in the rumen only at very short retention times with only a slight effect at very moderate to long solid retention times.

Intake

Level of feeding, method of preparation and type of food have an influence on the rate of passage of food through the digestive tract (Leaver et al., 1969). Depressions in digestibility of a low forage diet of 32% forage and 68% concentrate and a high forage diet of 83% forage and 17% concentrate given to dairy cattle fed at maintenance and *ad libitum* were measured by Colucci et al. (1982). Average dry matter digestibilities of the low forage and the high forage diets at maintenance were 74.73 and 68.59%; these decreased to 68.42 and 65.38% for the cows fed *ad libitum*. Retention times in the digestive tract for forage and concentrates decreased as level of intake increased, but within both diet and intake forage moved slower than concentrate in both the reticulorumen and the lower tract. These results were similar to those earlier obtained by Leaver et al. (1969) who reported a decline in crude fibre digestibility with an increase in intake of diets containing ratios of 1:1 and 1:4 hay to concentrate fed to castrated male sheep. The increase in the level of feeding caused a decrease in the mean retention time of hay particles stained with brilliant green. Although the retention time of markers in the digestive tract of sheep fed Lucerne chaff decreased by approximately 50% as their allowances increased from 400 to 1300 g per day, the apparent organic matter digestibility disproportionately decreased from 0.657 to 0.631 (Grovum and Williams, 1977).

Several studies have investigated the effects of intake on rumen turnover and particle distribution. For instance Colucci et al. (1984) compared the relationships among level of intake, rate of passage of digesta and proportions of forage to concentrate in the diet between sheep and cattle. For all diets cows at high levels of intake digested less organic matter and had faster liquid turnover rates than sheep, but the passage rates of particulate matter were similar in both species. Doubling intake of a high concentrate did not affect rumen volume in both species but rumen flow rates were doubled and this caused a doubling in the rate of passage of only fluid. Deswysen and Ellis (1988) evaluated the site and extent of neutral detergent fibre digestion, efficiency of ruminal digesta flux and faecal output as related to variations in voluntary intake and chewing behaviour in heifers. Their results suggested that heifers with higher voluntary intake had greater efficiency of chewing, flux of NDF through the reticulo-omasal orifice and less potentially digestible NDF digestion in their forestomach with compensatory greater potentially digestible NDF digestion in their large intestine. In addition, the efficiency of digesta flow per reticular contraction increased at high intake levels.

Okine and Mathison (1991) further investigated how changes in attributes of reticular contractions were related to changes in passage of digesta from the reticulorumen of cattle fed a forage-based diet at maintenance level and three levels above maintenance. Their results suggested that the flow of neutral detergent fibre from the reticulorumen with increasing dry matter intake were related primarily to the duration and amplitude, and not frequency, of reticular contractions. Therefore changes in duration and amplitude of reticular contractions were more important determinants of digesta passage from the reticulorumen than frequency. However, Dado and Allen (1995) observed an increase in the number of reticular contractions as the fractional passage rate of NDF from the rumen increased in dairy cows receiving a high fibre diet and rumen inert bulk, to compensate for increased rumen fill and to maintain intake.

The effects of feeding level on faecal particle size and ruminal particle distribution is variable. Bae et al. (1981) fed hay at four levels of intake to dairy cows. Their results suggested that increasing level of feeding had no effect on the size of digesta particles in the reticulorumen and faeces. Waghorn et al. (1986) also reported that there was no significant change in abomasal particle weight distribution in sheep when their allowance of chopped alfalfa hay was increased from 700 to 1020 g/day. However, Luginbuhl (1990) found that increasing level of intake resulted in a linear increases in rumen upper strata and faecal particle sizes in their investigation of changes in ruminal and faecal particle weight distribution of steers fed coastal bermudagrass (*Cynodon dactylon*) hay at four intake levels. An increase in the size of particles in the dorsal sac with an increase in intake was suggested by the results of Evans et al. (1973) to monitor changes in some physical characteristics of the digesta in the reticulorumen of cows fed three levels of pasture hay once daily.

Processing

Voluntary intake of poor quality forages seems to be reduced by the length of time the digestive tract takes to clear them, but chopping or grinding and pelleting may improve their intake (Allen, 1996), provided nutrient deficiencies do not limit intake (Minson, 1982). For instance, de Vega et al. (2000) investigated the effects of the form of feeding of lucerne hay and its feeding frequency on voluntary intake, digestibility, feeding behaviour and marker kinetics in ewes. Grinding and pelleting resulted in higher intake and a reduction in dry matter digestibility. Processing forages by chopping or grinding causes an increase in intake and fill of digesta in the reticulorumen but the resulting increased energy intakes may not be completely available to the animal because decreased retention time of digesta may reduce energy digestibility (Weston and Kennedy, 1984). Blaxter et al. (1956) compared the digestibilities and retention times of dried grass in the long form, the same grass medium-ground (in a hammer mill through .25 inch sieve) and cubed and finely ground (twice through a .00625 inch sieve) and cubed in the digestive tracts of sheep fed at different levels of intake. The results indicated that the cubes of dried grass passed through the digestive tract more quickly and had a lower digestibility than the long material. Increasing feeding level resulted in an increase in the passage of food and a fall in its digestibility. These results led them to the conclusion that the method of preparation modified the rate of passage of through the gut and that this rate was the determinant of its digestibility. This conclusion was later corroborated by Tetlow and Wilkins (1974) who observed a faster rate of passage of ground compared to chopped tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*) in the digestive tracts of sheep. By contrast Faichney (1983) found that grinding and pelleting of lucerne (*Medicago sativa*) hay increased the mean retention times of solutes and of particle associated marker in the rumen of sheep.

Plant structure

Plant structural characteristics exert an influence on its behaviour in the gut and therefore impact on its passage rate and digestibility (Wilson et al., 1989). Laredo and Minson (1975) investigated the effect of grinding and pelleting and chopping on the voluntary intake and digestibility of leaf and stem fractions of three grasses offered to wethers. Voluntary intake of chopped leaf was 34% higher than that of chopped stem but their digestibilities did not differ significantly. Chopped leaf was also retained almost 6 h shorter than chopped stem. Grinding and pelleting increased the voluntary intake of the leaf fraction by 88% and the stem fraction by 60%. The conclusions drawn from the study that the higher intake of the leaf fraction was attributable to its shorter retention time in the reticulorumen and that it was the physical more than the chemical characteristics that determined the different rates of passage.

However, there is evidence that the rates of passage and thus digestibilities of plant species are influenced by their structural organisation, including their chemical characteristics (Wilson, 1993). For instance, Baumont et al. (1990) compared the alimentary behaviour of alfalfa (*Medicago sativa)* and orchardgrass (*Dactylis glomerata)* hays in sheep and observed a much higher voluntary intake of alfalfa than orchard grass (1757 versus 1099 g/day). Jung and Allen (1995) attribute the higher intake for alfalfa to a lower filling effect of alfalfa neutral detergent fibre because of its faster degradation and passage from the rumen than grass. Similarly, Campling et al. (1961), comparing the consumption of hay and straw by cows and its residence time in the alimentary tract, found that the intake of hay was more than twice that of straw and attributed that to the longer retention time of straw in the digestive tract. Wilson et al. (1989) compared particle size reduction of the leaves of a tropical grass, green panic (*Panicum maximum*) and Italian ryegrass (*Lolium multiflorum*) due to chewing during eating and to varying times of digestion by cattle. Results suggested that ryegrass was more digestible than green panic from within 6 h of digestion up to 96 h. After 3 weeks of the digestibility of ryegrass was still higher than that of green panic but the proportion of cell wall that was ultimately undigested was the same for both grasses.

Physiological factors

The movement of digesta in the digestive tract is also modulated by animal factors and these include animal response to lactational, gestational and climatic changes, which may increase or decrease their rate of passage.

Pregnancy and lactation

Voluntary dry matter intake by ruminants decreases during late pregnancy (Forbes, 1986). Pond et al. (1984) investigated the rate of passage of chromium mordanted fibre and Co-EDTA in the gastrointestinal tract of pre- and postpartum ewes fed Coastal bermudagrass (*Cynodon dactylon*) pellets *ad libitum*. They reported mean residence time reductions of 3.5 h for liquid and 6 h for solid digesta fractions postpartum compared to prepartum. Their results suggested that although the increase in passage rates could be attributed to increased intake postpartum, another mechanism could have contributed to the difference. The effects of early and mid pregnancy of sheep on the passage of a mixture of chopped lucerne *(Medicago sativa)* hay and wheaten chaff given at four levels of feeding were determined by Graham and Williams (1962). Similar to the results of Pond et al. (1984), the mean retention time of solid digesta increased as pregnancy advanced, by 1 to 1.5 h per 100 g increase of concepta. Intake had a positive relationship with digesta flow rate, but these researchers also entertained the idea of a possible change of metabolism during pregnancy, which could cause an increase in the rate of flow of digesta.

Weston (1988) studied passage rates at a constant rate of intake (1100 g/day) of a 1:1 mixture of chopped lucerne (*Medicago sativa*) and wheaten hays in pregnant sheep bearing twin or single foetuses and in ewes suckling a single lamb during early lactation. Both late pregnancy and early lactation caused an increase in the passage rates in the gastrointestinal tract with a reduction in organic matter digestion, but the effects of late pregnancy were more pronounced. The ewes with twins showed similar passage rates as the ewes with singles. Ehle et al. (1984) observed a quadratic rumen turnover rate as lactation progressed from dairy cows fed at least 25% grain and different amounts of alfalfa and bromegrass *(Bromus inermis)* haylage *ad* *libitum*. They also found a poor correlation between intake levels and rumen turnover rates, suggesting the involvement of another mechanism for regulation of rumen turnover rates in lactating cows, besides level of feed intake.

Climate

Climatic factors seem to affect intake and thus digesta passage rates and digestibility. For example, Kennedy et al. (1976) found cold exposure to reduce mean retention times and dry matter digestibility of pelleted bromegrass (*Bromus spp.*) in the reticulorumen of shorn sheep. Warren et al. (1974) compared digestibility and ingesta passage in cows fed alfalfa (*Medicago sativa*), tall fescue (*Festuca arundinacea*) and orchardgrass (*Dactylis glomerata)* under 18 ^{0}C and 32 ^{0}C ambient temperatures. Results suggested that digestible dry matter intake, water intake and mean retention time increased as temperature increased. There was however no relationship between voluntary dry forage intake and mean retention time and the authors concluded that other factors like gut fill may be more limiting than mean retention time in relation to forage intake.

Use of markers

The most commonly used methods for measuring passage rates involves administration of indirect markers (Faichney, 1993). Criteria for the ideal marker have been suggested and include inertness, noninterference with the digestive or physiological processes, physical similarity to or intimate association with the fraction to be marked, recoverability and easy quantification (Kotb and Luckey, 1972; Faichney, 1993). However, none of the markers in use today meets all the desirable characteristics (Dove and Mayes, 1991). Markers may be divided into internal and external indicators (Van Soest, 1982).

Internal markers

Internal markers like lignin, chromogens and faecal nitrogen may be useful as digestibility markers, but they are not suitable for passage rate studies (Van Soest, 1994). Although chromogens are relatively inabsorbable and not utilized by rumen microbes, they are degraded into substances with different properties to the original (Van Soest, 1994). The recovery of faecal nitrogen is variable because of the contribution of bacterial nitrogen, which can cause measurement errors (Van Soest, 1982). Lignin has been extensively used as a marker in digestibility studies, but microbial activities in the rumen and lower gut may degrade it or alter it, making quantification difficult (Fahey and Jung, 1983). Analytical techniques also differ in their estimation of plant lignin content (Fahey and Jung, 1983). Isotopic labelling of plant cell walls can provide a good method of measuring passage rates because if done properly the labelled material has the same characteristics as the diet (Van Soest, 1994). Their wide use is hampered by the need to grow the labelled plant in a light chamber (Uden et al., 1980) and the potential hazards associated with exposure to radioactivity.

External markers

Feed stained with dye may be an ideal marker in that its physical and chemical characteristics are not much different from those of the particulate fraction of the diet but their quantification is difficult due to incomplete recovery (Van Soest, 1988; Uden et al., 1980). Incomplete recovery may be due to digestion and can lead to inaccurate estimation of passage rates. Disintegration of stained particles as a result of rumination and digestion magnify the problem of incomplete recovery because they increase the amount of fines which may not be identifiable and counted (Uden et al., 1980). This method may hold promise if quantification can be improved.

Plastic particles have been use to provide information about rumination (desBordes and Welch, 1984) and selective retention of particles of different characteristics (Kaske and Engelhardt, 1990). They can be manufactured to different colour, size, shape and density requirements (Welch, 1990). Their quantification is relatively simple and does not require sophisticated, expensive equipment but it is labour intensive (Welch, 1990). However, because plastic particles do not undergo the same changes like hydration and specific gravity increase that occur in feed particles in the gut, data obtained from their use cannot provide absolute rates about feed particles (Uden et al., 1980). The same disadvantages are true of various other similar markers like rubber pieces, glass beads and ball bearings.

Heavy metal ions such as those of Cr and rare earth elements have been attached to fibre particles and used as solid digesta markers (Uden et al, 1980). Cr forms stable plant cell wall and protein complexes that are stable in *in vitro* digestion processes

(Uden et al., 1980). Because of the tightness of its attachment, Cr reduces the digestibility and increases the density of the material it is intended to label (Ehle, 1984; Van Soest, 1988). Rare earths, although variable in degree of attachment, can reduce digestibility and estimates of passage rates obtained through their use may be influenced by the method of attachment (Mader, 1984). Another problem with rare earth metals is that their wide reactivity with proteins and carbohydrates could result in the formation of insoluble complexes likely to move independently of the feed particles they were intended to mark (Van Soest, 1988).

One of the commonest methods of estimating intake of grazing animals makes use of digestibility and faecal output relationship (McMeniman, 1997). Digestibility can be estimated using several indirect methods, including internal markers like chromogen, fibre and methoxyl. Faecal output can be determined by total faecal collection, but this method is not commonly used because it is cumbersome for the collector and may interfere with normal grazing behaviour. Indigestible markers used to estimate faecal output, instead of total faecal collection. Although a number of substances have been used as indigestible markers for faecal output estimation, the most commonly used is chromic oxide dosed manually daily in fixed quantities or by using controlled release devices (Chen et al., 1999; McMeniman, 1997). However, intake estimates derived by using this method have a certain degree of inaccuracy because they do not take into account individual differences in digestibility due to physiological status or even constitutions as they use one digestibility estimate for all animals (Dove and Mayes, 1991). The alkane technique does not suffer from this problem and seems to be an improvement over chromic oxide and digestibility method.

Estimation of digestibility

Estimation of digestibility is difficult because of the difficulty of obtaining a digestibility estimate that is representative of the test animals because it is almost impossible to obtain a representative sample of what animals eat. The use of oesophageal fistulated animals tries to address the problem of obtaining feed samples that are representative of what is consumed by test animals. However, extrusa samples represent only what the fistulated animals consumed during the observational period and do not necessarily represent the diet selected by the test animals over a long period of time (Mayes and Dove, 200). The most widely used method is the two-stage method described by Tilley and Terry (1963) or its derivatives. In addition to not accommodating digestibility as a function of intake dry matter amount, this method derives digestibility estimates that are used for all test animals and as such does not cater for individuality in digestibility. Also, the calibration animals may not be in the same physiological status as the test animals.

As an improvement over the traditional methods many components of plants have been explore for use as internal markers for digestibility, including those discussed above, but most have not been satisfactory. Odd-chain alkanes which are also components of plant cuticular wax are used as internal markers for estimating digestibility, together with a dosed even-chain alkane that is used as a faecal output marker to unbiased estimates of intake, if the two alkanes have the same recovery rates (Mayes and Dove, 2000).

CHAPTER 3

Introduction

The nutrient supply to grazing animals is determined by the quantity and composition of ingested forage. However, these variables are difficult to measure particularly for animals grazing mixed species swards. Historical methods for estimating the species composition of consumed herbage are either laborious or imprecise in distinguishing between species in an herbage mixture (Dove and Mayes, 1991). Since their first use as markers for estimating herbage intake by grazing animals in the mid 1980s, n-alkanes, which are saturated hydrocarbons found in the cuticular wax of most plants, have been used to quantify the species composition of herbage mixtures.

Dove (1992), Newman et al. (1995) and Mayes et al. (1994) have presented n-alkane-based methods of estimating the composition of a consumed diet, which are essentially mathematical derivations of the best match between the n-alkane concentrations in the diets on offer and the faeces, corrected for losses in the gut (Hameleers and Mayes, 1998). In addition, if the grazing animals are dosed with known quantities of another n-alkane, usually an even-chain n-alkane, used as an external marker, the concentrations of n-alkanes in the herbage and faeces of the dosed animals can also be used to estimate dry matter intake without additional chemical analyses (Dove and Mayes, 1996). However, cyclic variations of dosed even-chain n-alkanes, which could compromise the accuracy of the technique have been reported (Dove and Mayes, 1991, Dillon and Stakelum, 1990). Also, the most widely used method of administering alkanes to animals is by dosing twice daily with either paper pellets or gelatin capsules, yet dosing once daily would have the advantage of reduced animal handling, but it can cause variation with time in the concentration of the dosed alkane (Dove and Mayes, 1991). These sources of variation can affect the accuracy of the alkane methodology in estimating the diet composition and amount of the herbage consumed by grazing animals.

This study was thus designed to assess under controlled conditions the consequences of possible sources of error on the accuracy of the n-alkane technique in estimating the composition and dry matter intake by animals given diet mixtures. Three experiments were conducted to investigate the following possible sources of error: consequences of amount of food eaten as a function of animal mature size, diurnal variation in n-alkane concentration in the gut and whether this variation is influenced by food allowance (*ad libitum* or restricted feeding), dosing once daily versus twice daily.

The experiments were as follows:

Experiment 1was designed to compare the estimates derived from n-alkane concentrations in herbage and in the faeces of lambs at 30 and 45% of projected mature weights with direct measurements of:

a) grass dry matter intake when pelleted grass was offered alone.

b) lucerne dry matter intake when pelleted lucerne was offered alone.

c) the proportion and dry matter intake of lucerne and grass in selected diet when pelleted grass and pelleted lucerne were offered together as a choice.

Experiment 2 sought to investigate the diurnal pattern of excretion of the faecal n-alkane ratios of tritriacontane (C_{33}) to dotriacontane (C_{32}) over a 24 h period when lambs are fed *ad libitum* or a restricted amount of feed and dosed twice daily with dotriacontane ($C_{32.}$).

Experiment 3 sought to compare faecal alkane ratios of tritriacontane (C_{33}) to dotriacontane (C_{32}) between lambs dosed once daily and twice daily with dotriacontane (C_{32}).

Materials and methods

The study was carried out indoors at the Scottish Agricultural College Research Farm, Bush Estate, Edinburgh. The three experiments were carried out between early June and late July 1998.

Experiment 1

This experiment was carried out in two phases: the first phase began in early June when, on average, the lambs had reached 30% of their estimated mature weight and the second phase began in late July when, on average, they had reached 45% of their estimated mature weight. Following weaning at 20% mature weight and a two-week adjustment period, the lambs were individually penned on slatted floors until they had reached 30% of their estimated mature weight for the start of phase 1 of the trial. In each phase 36 lambs were used in a factorial design with 2 breeds [Suffolk (S) and Scottish Blackface (B)], two sexes (male and female) and three feed treatments [pelleted lucerne (*Medicago sativa*) or pelleted ryegrass (*Lolium* spp) alone or both as a choice]. Each treatment was replicated three times. Mean weights for the lambs used in phase one were 30kg for S females and 39kg for S males, and 21kg for B females and 28kg for B females. Mean weights for the lambs used in phase two were 48kg for S females and 59kg for S males, and 37kg for B females and 44kg for B females.

For direct measurement, a pre-weighed amount of food sufficient to last one week was prepared for each lamb and placed in a reservoir. A sufficient amount was offered from the reservoir twice daily at 08h30 and 15h30 to ensure its *ad libitum* availability through the day and night. Refusals were removed from each pen weekly and weighed. For the n-alkane method, each lamb was dosed over a 12-day period twice daily at 08:00 and 16:00 with 0.1g of n-dotriacontane (C_{32}) impregnated into a small cellulocotton filter. From day eight faecal grab samples were collected twice daily at 08:00 and 16:00 for five days from each lamb. The samples were oven dried at 60^0 C for 48 hours and then ground. For each lamb, the two faecal samples from each day were bulked and an aliquot extracted as described by Mayes et al. (1986), with the modifications that duplicate 0.5g samples were directly heated in ethanolic potassium hydroxide and 7ml of heptane were used as the solvent.

Duplicate 1g samples from each of the two diets were extracted the same way as faeces except that about 50% more solvent and water than in faecal extraction were used. The final eluates were injected onto a capillary column (HP.5 Crosslinked Phenyl Methyl Silicone, 50m x 0.32mm x 0.17μm) in a model HP6890 gas chromatograph fitted with a flame ionization detector and linked to a PC run by an HP Chemstation software. The oven temperature programme was initially 65^0C and then rose to 300^0C at 10^0C/min where it was held for 20 min. The temperatures of the injection and detector ports were 325^0C and 350^0C, respectively. The flow rate of the carrier gas (N_2) was 33.4ml/min. and that of the makeup gas (N_2) was 24ml/min. The ratio of the peak areas of the analysed n-alkanes to that of the internal standard (C_{34}) was used to calculate n-alkane amounts in the samples. Identification of the different n-alkanes was made based on the relative retention times of known standards.

Several cellulocotton filters from the batch that was used for dosing the lambs were extracted to determine the average amount that was adsorbed onto each filter. The method involved slicing three pellets and boiling them for 1h under reflux in 100ml of heptane after adding 100mg of internal standard (C_{34}), (Mayes unpublished protocol). After partial cooling to about 60^0C an aliquot was taken and analysed by gas chromatography the same way as the faecal and feed samples.

For the lambs fed on grass or lucerne alone, dry matter intakes were estimated from the concentrations of n-alkanes (C_{32} and C_{33}) in the faeces and the consumed feed according to the method described by Mayes et al. (1986). For the lambs offered lucerne and grass as a choice, several different estimates of the proportion of lucerne in the selected diet derived by a least-squares method similar to that described in Newman et al. (1995) were compared against the observed proportion of lucerne. The different estimates were derived by using four (C_{27}, C_{29}, C_{31} and C_{33}), and different combinations of three or two alkanes, with the recovery rates published by Mayes et al. (1986) and by using four and two alkanes (C_{31} and C_{33}) with the recovery rates adopted from Dillon and Stakelum (1990) in the least squares programme. (Figure 6). In all cases the least-squares solutions were constrained to non-negative values. The recovery rates reported by Mayes et al. (1986) were 0.713, 0.745, 0.854 and 0.891 for C_{27}, C_{29}, C_{31} and C_{33}, respectively and those of Dillon (1993) cited by Hameleers and Mayes (1998) were 0.753, 0.767, 0.826 and 0.838 for C_{27}, C_{29}, C_{31} and C_{33}, respectively. The combination of the two alkanes, C_{31} and C_{33} used with the Mayes et al. (1986) recovery rates gave the closest estimates to the observed lucerne proportions and these estimates were therefore adopted and used in subsequent calculations. The total DMI of lucerne and grass by the lambs were calculated by adapting the Mayes et al. (1986) formula to use diet n-alkanes weighted according to the diet proportion estimates derived from the least-squares solutions as follows:

$$\text{Total DMI} = \frac{(F_{C33}*D_{C32}/F_{C32})}{\{(P_L*L_{C33}+P_G*G_{C33})-[F_{C33}*(P_L*L_{C32}+P_G*G_{C32})/F_{C32}]\}}$$

where F_{C33}, L_{C33} and G_{C33} are concentrations (mg/g DM) of C_{33} in the faeces, lucerne and grass; F_{C32}, L_{C32} and G_{C32} are concentrations of C_{32} in the faeces, lucerne and grass; D_{C32} is the daily dose of C_{32}; P_L and P_G are the proportions of lucerne and grass derived from the least squares method. The amount of lucerne or grass eaten is then derived by subtracting the relevant proportional amount (based on the least squares solution) from the total amount of the consumed diet.

For all the treatments the estimated and the observed mean daily dry matter intakes of lucerne and/or grass for each lamb were compared by regressing the estimated on the observed to test the reliability of estimates at both stages of maturity. Linear regressions of the estimated on the observed proportions of lucerne in the diet at both stages of maturity were also compared. The expectation from the fits of the linear regression of the estimated on the observed intake (or diet proportion) values was that the intercept would be 0 and the slope 1 if the n-alkane method accurately estimated the observed scenarios. All the linear regressions were performed using the Minitab statistical package (1993).

Experiment 2

One week before dosing, six male Suffolk lambs at 45% mature size were each given a daily allowance of 1030 g of pelleted grass. One lamb did not consume all its daily allotment so that over the experimental period it consumed on average about 750 g per day. The aim of the feeding restriction was to ensure that the daily feed allowance was eaten in 1-2 hours. These lambs were dosed as described in experiment 1. Faecal samples were also collected as described in experiment 1, except that on the third day of the faecal collection period, a faecal sample was obtained from each lamb at 4 hourly intervals over a 24 hour period. In addition, faecal samples were collected from 6 Suffolk lambs on the *ad libitum* grass diet also at 45% mature size (from experiment 1) at the same intervals and on the same day as for those lambs on restricted feeding. Samples were oven dried and ground as described in experiment 1, but each sample was analysed individually (as opposed to bulking the daily samples as in experiment 1). The ratios of the amount of dotriacontane (C_{32}) to tritriacontane (C_{33}) in the faeces of the restricted and *ad libitum* fed lambs were compared over the 24 hour when sampling was done at 4 hourly intervals. The collected data were repeated measures and did not satisfy the requirements for compound symmetry and were analysed as a split-plot after applying a Greenhouse-Geisser correction factor (Rao, 1998), with animals as the main plots and sampling times as the subplots using Genstat statistical package (1998).

Experiment 3

At 45% mature size, six Suffolk male lambs, three offered pelleted grass *ad libitum* and three offered pelleted lucerne *ad libitum* were given 2 x 0.1g of dotriacontane once in the morning (08:00) from the beginning to the end of the dosing period. Faecal samples were collected in the same way as described in experiment 1, except that the two samples collected in the morning and afternoon were bulked after drying and milling to make a daily composite sample for each animal. Additional samples from six Suffolk lambs that were dosed in the morning and afternoon (experiment 1) were also bulked in the same way to make daily composite samples. The composite samples were oven dried and ground as described in experiment 1, but each composite sample was analysed individually. The ratios of the amounts of dotriacontane (C_{32}) to tritriacontane (C_{33}) in the faeces of the lambs that were dosed once daily and twice daily were compared. Because the experimental data were repeated measures and did not satisfy the requirements for compound symmetry, they were analysed as a split-plot after applying a Greenhouse-Geisser correction factor (Rao, 1998), with the number of dosings as the main plots and sampling times as the subplots using Genstat statistical package (1998).

Table 1. Concentrations (mg/g DM) of odd-chain n-alkanes in the diets

Diet	Alkanes			
	C_{27}	C_{29}	C_{31}	C_{33}
Lucerne	0.0328	0.1289	0.2645	0.0174
Ryegrass	0.0334	0.1729	0.2164	0.0674

Results

The concentrations of the odd-chain n-alkanes of the diets used in this study are presented in Table 1. The concentrations of C_{27}, C_{29} and C_{31} of the ryegrass were about 50 percent higher than those reported by Hameleers and Mayes (1998) but similar to those reported by Newman et al. (1995). The concentration of C_{33} was much lower than those found by either of the above studies. The concentrations of all the n-alkanes in the lucerne were different to those obtained by Dove (1992). There were 1.5 times more C_{27} and C_{29} found in this study than in Dove's (1992). C_{31} and C_{33} concentrations were more than 2 times higher in this study.

Experiment 1

The means of the observed and estimated dry matter intake amounts when the lambs were fed on either lucerne or grass are alone, and the means of the estimated and observed proportion of lucerne and dry matter intake amounts of grass and lucerne in the selected diet when the lambs were fed both diets as a choice are given in Table 2. The results suggested that there was a general agreement between the measured quantities and those estimated using the alkane technique across all the three diet treatments and the two mature sizes. However, at 30% mature size, for the lambs fed on grass only, dry matter intake was slightly overestimated and for those fed on lucerne and grass as a choice, the dry matter intake of lucerne was underestimated by the alkane technique. There was also an increase in dry matter consumption by the lambs from 30 to 45% mature size on all the diet treatments. The observed dry matter intake of grass increased by almost 800g, while the estimated quantity increased by just over 600g from 30 to 45% mature size. The dry matter intake of lucerne increased by over 800g between the two lamb sizes and the observed and estimated quantities were similar. The combined dry matter intake of grass and lucerne increased by just over 900g and similar margins of increase were reflected in both measured and estimated intake amounts, but as mentioned above, there was a discrepancy between the observed and estimated dry matter intake amounts of lucerne at 30% mature size.

The results of regressing the estimated quantities generated by the alkane technique on the observed quantities for the three diet treatments and the two animal mature sizes are tabulated in Table 3. The slopes of most of the regression lines relating the estimated and the observed amounts were not significantly different from 1 and most of their intercepts were not significantly different from zero, which suggests a close agreement between the estimated and measured amounts.

The relationship between the observed dry matter intake of grass and the estimated dry matter intake of grass using the n-alkane technique for the lambs at 30% and 45% mature sizes is presented graphically in Figure 1. At 30% mature size there was a linear relationship (R^2=.78, $p<0.001$) between the observed and estimated daily dry matter intake of grass, albeit with a slope that is significantly different from 1 and an intercept that is significantly different from zero (Table 3). The regression equation describing the relationship is: $Y_{DMI} = 450 \ (SE \pm 156) + 0.688 \ (SE \pm 0.110) \ X_{DMI}$, where Y_{DMI} is the estimated dry matter intake and X_{DMI} is the observed dry matter intake; the r. s. d. was 116 g/day. Although the technique slightly underestimated the intake of grass by a few lambs and overestimated the intake of one at 30% mature size, the average of the estimated (1402; SE=65.7) was only 1.3% higher than the average of the observed (1384; SE=84.3) dry matter intake of grass. At 45% mature size there was a linear relationship (R^2=0.90, p<0.001) between the observed and estimated daily dry matter intake of grass such that: Y_{DMI}= 175 (SE±189) + 0.850 (SE±0.086) X_{DMI}, where Y_{DMI} is the estimated dry matter intake and X_{DMI} is the observed dry matter intake; the r. s. d. was 109 g/day (Table 3).

Table 2. Comparison of the means of the estimated and observed dry matter intake and lucerne proportion of the diets of the lambs at 30 and 45% mature sizes

Diet Treatment	Number of Lambs	Mature Size (%)	Mean livemass (Kg)	Measured Parameter	Observed Quantity	Standard Error	Estimated Quantity	Standard Error
Grass alone	13	30	30.2	[1]DMIG	1383.8g	84.3	1401.9g	65.7
	13	45	44.5		2177g	102	2026.3g	91.4
Lucerne alone	11	30	29.3	[2]DMIL	1761g	141	1853g	148
	11	45	49.9		2635g	217	2671g	241
Grass and Lucerne as a choice	12	30	29.5	[3]PROPL	0.596	0.0622	0.572	0.0578
				[1]DMIG	610.8g	79.4	632.9g	72.5
				[2]DMIL	1018g	160	926g	129
	12	45	48.4	[3]PROPL	0.659	0.0380	0.653	0.0394
				[1]DMIG	858g	111	836g	104
				[2]DMIL	1694g	196	1627g	181

[1]DMIG= Dry matter intake of grass
[2]DMIL= Dry matter intake of lucerne
[3]PROPL= Proportion of lucerne in the diet

Table 3. Results of regressing estimates generated by the alkane method on observed diet proportions and dry matter intakes for the various diet treatments

Diet Treatment	Mature Size (%)	Number of Lambs	Regression Equation	RSD	R^2	[a]Slope Significance	[b]Intercept Significance
Grass	30	13	Y_{DMIG}= 450 (SE±156) + 0.688 (SE±0.110)X_{DMIG}	116	0.78	*	*
	45	13	Y_{DMIG}= 175 (SE±189) + 0.850 (SE±0.086) X_{DMIG}	109	0.90	NS	NS
Lucerne	30	11	Y_{DMIL}= 51 (SE±130) + 1.02 (SE±0.071) X_{DMIL}	106	0.95	NS	NS
	45	11	Y_{DMIL} = -104 (SE±313) + 1.05 (SE±0.115) X_{DMIL}	262	0.90	NS	NS
Grass and Lucerne	30	12	Y_{PROPL}= -0.0126 (SE±0.0318) + 1.06 (SE±0.0526) X_{PROPL}	0.0350	0.98	NS	NS
	45	12	Y_{PROPL}=0.0581(SE±0.0616) + 0.919 (SE±0.0925) X_{PROPL}	0.0419	0.91	NS	NS
	30	12	Y_{DMIG}= 88.8 (SE±41.5) + 0.891 (SE±0.0631) X_{DMIG}	57.5	0.95	NS	NS
	45	12	Y_{DMIG}= 84.1 (SE±94.4) + 0.876 (SE±0.101) X_{DMIG}	129	0.88	NS	NS
	30	12	Y_{DMIL}= 122 (SE±54.3) + 0.790 (SE±0.0473) X_{DMIL}	87.0	0.97	**	*
	45	12	Y_{DMIL}= 85.0 (SE±94.4) + 0.911 (SE±0.0521) X_{DMIL}	117	0.97	NS	NS

Y_{DMIG}= Estimated grass dry matter intake
X_{DMIG}= Observed grass dry matter intake
Y_{DMIL}= Estimated lucerne dry matter intake
X_{DMIL}= Observed lucerne dry matter intake
Y_{PROPL}= Estimated proportion of lucerne in the diet
X_{PROPL}= Observed proportion of lucerne in the diet
NS= Not significant; *= significant ($p<0.05$); **= significant ($p<0.01$)
[a,b]for agreement between the estimated and observed values the slope of the regression line should not be significantly different from 1 and the intercept should not be significantly different from 0

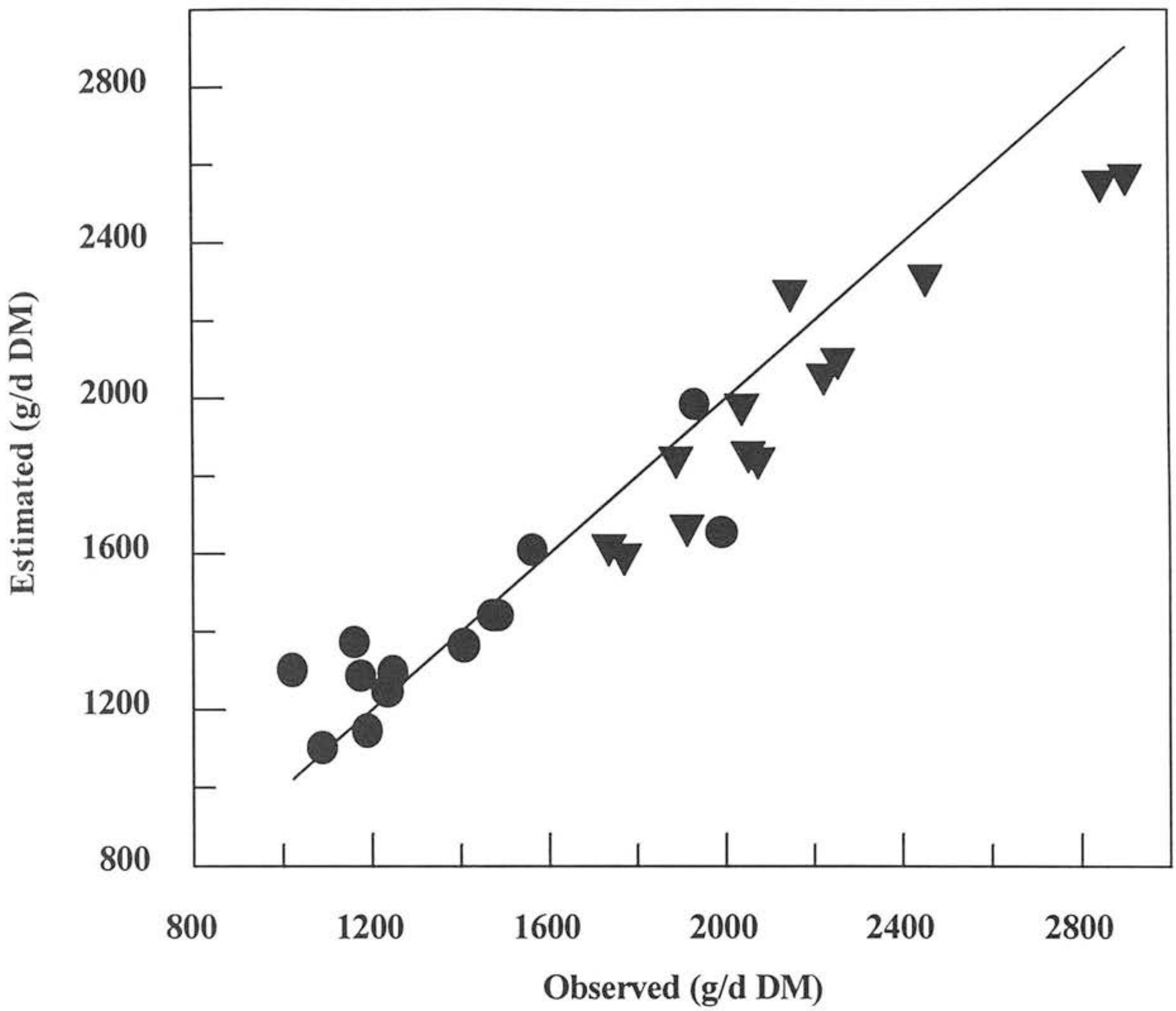

Figure 1 The relationship between observed and estimated dry matter intake for the lambs fed on grass alone at 30% and 45% mature sizes *(n=13)*. ●, 30% mature size; ▼, 45% mature size and the solid line indicates the line of equality.

In Figure 2 the relationship between observed and estimated dry matter intake for the lambs fed on lucerne alone is shown. At 30% mature size there was a linear relationship (R^2=0.95; $p<0.001$) between the observed and the estimated daily dry matter intake of lucerne such that: Y_{DMI}= 51 (SE±130) + 1.02 (SE±0.071) X_{DMI}, where Y_{DMI} is the estimated dry matter intake and X_{DMI} is the observed dry matter intake with r. s. d. of 106 g/day. At 45% there was also a linear relationship (R^2=0.90; p<0.001) between the observed and the estimated daily dry matter intake of lucerne and this relationship is described by the regression equation: Y_{DMI} = -104 (SE±313) + 1.05 (SE±0.115) X_{DMI}, where Y_{DMI} is the estimated dry matter intake and X_{DMI} is the observed dry matter intake with r. s. d. of 262 g/day.

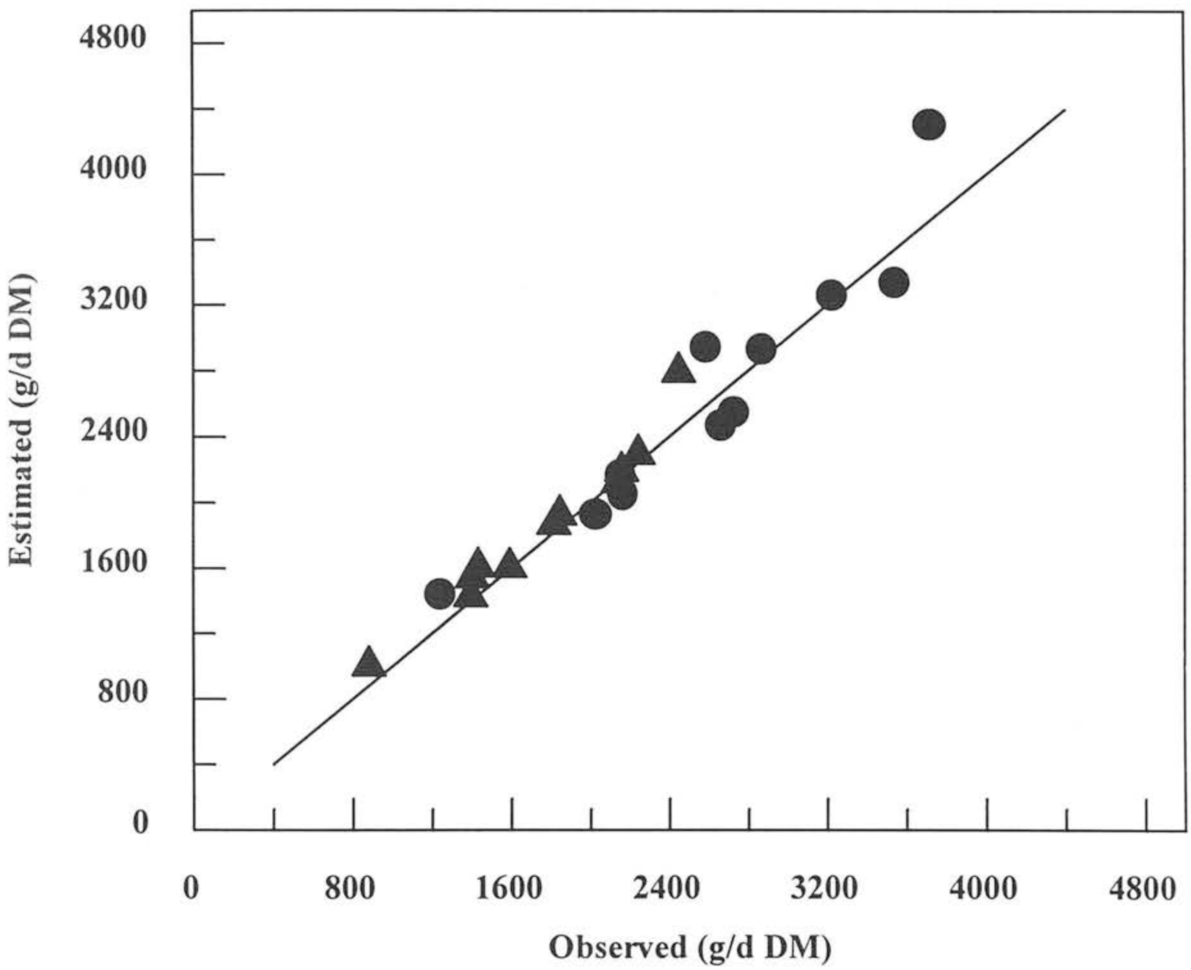

Figure 2 The relationship between observed and estimated dry matter intake for the lambs fed on lucerne alone at 30% and 45% mature sizes *(n=11)*. ●, 30% mature size; ▼, 45% mature size and the solid line indicates the line of equality.

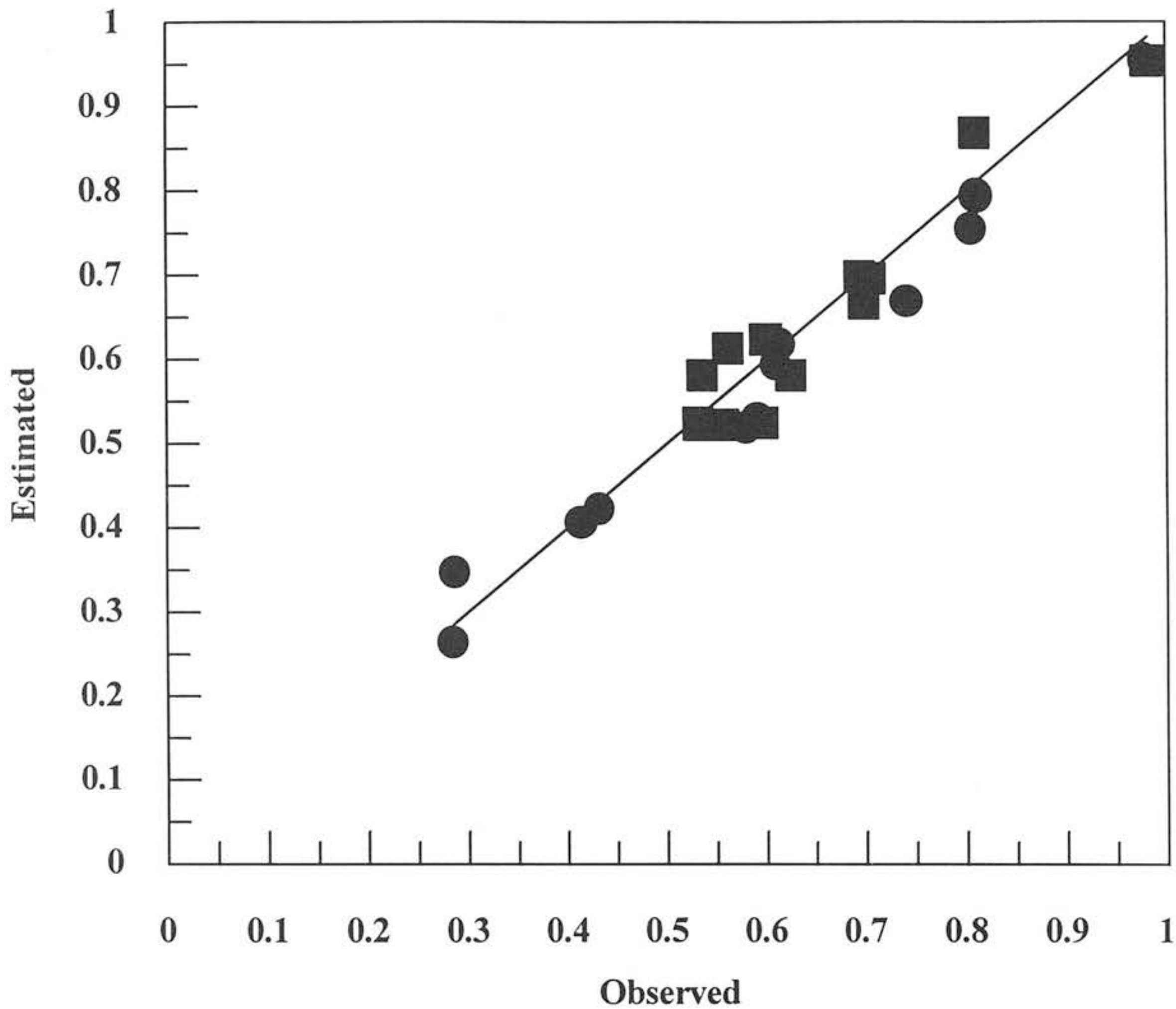

Figure 3 The relationship between estimated and observed proportion of lucerne in the diet of each of the lambs offered pelleted lucerne and pelleted grass as a choice *(n=12)*. ●, 30% mature size; ■, 45% mature size and the bold line indicates the line of equality.

Figure 3 shows the relationship between the observed and the estimated proportion of lucerne in the diet selected by the lambs when they were fed on both lucerne and grass as a choice. At both 30% and 45% mature sizes, there was a linear relationship

(R^2=0.98; *p<0.001* and R^2=0.91; *p<0.001,* respectively) between the observed and the estimated proportion of lucerne in the diet. At 30% mature size the relationship is described by the regression equation: Y_{PROP}= -0.0126 (SE±0.0318) + 1.06 (SE±0.0526) X_{PROP}, where Y_{PROP} and X_{PROP} is the estimated and observed proportion of lucerne in the diet, respectively; the r. s. d. was 0.0350 At 45% the relationship is described by the equation: Y_{PROP}= 0.0581 (SE±0.0616) + 0.919 (SE±0.0925) X_{PROP}, where Y_{PROP} and X_{PROP} is the estimated and observed proportion of lucerne in the diet, respectively with r. s. d. of, as a proportion, 0.0419.

Linear relationships between the observed and the estimated daily dry matter intake of grass were observed at 30% and 45% mature size (R^2=0.95; p<0.001 and R^2=0.88; *p<0.001,* respectively), when the lambs were fed on pelleted lucerne and grass given as a choice (Figure 4). At 30% mature size the relationship was described by the regression equation: Y_{DMI}= 88.8 (SE±41.5) + 0.891 (SE±0.0631) X_{DMI}, where Y_{DMI} is the estimated dry matter intake and X_{DMI} is the observed dry matter intake with r. s. d. of 57.5 g/day. At 45% mature size the relationship was described by the equation: Y_{DMI}= 84.1 (SE±94.4) + 0.876 (SE±0.101) X_{DMI}, where Y_{DMI} is the estimated dry matter intake and X_{DMI} is the observed dry matter intake with r. s. d. of 129 g/day.

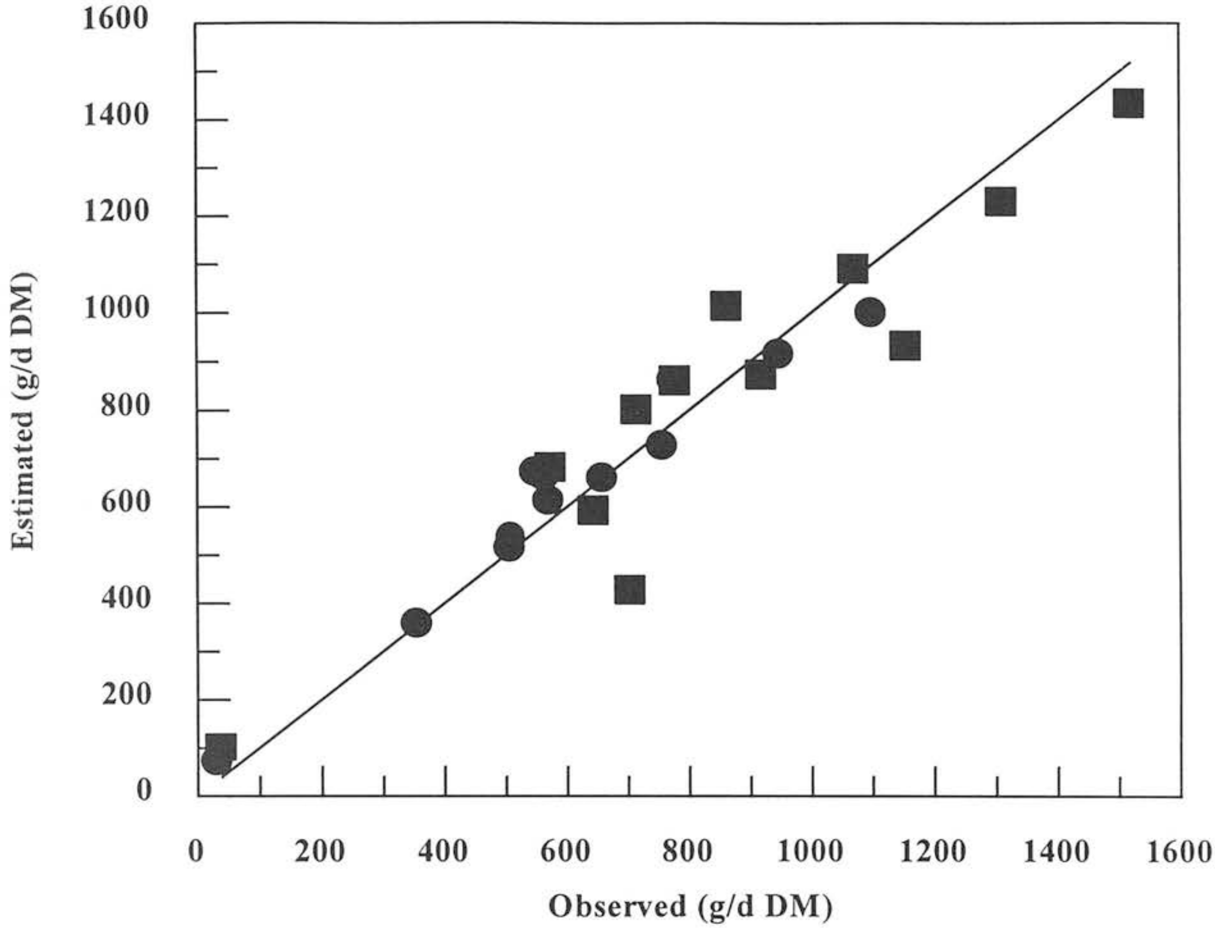

Figure 4 The relationship between the observed and the estimated amount of grass in the diet selected by each of the lambs given pelleted lucerne and pelleted grass as a choice at 30% and 45% mature sizes *(n=12)*. ●, 30% mature size; ■, 45% mature size and the bold line indicates the line of equality.

In Figure 5 the relationships between the observed and the estimated amount of daily dry matter intake of lucerne for each of the lambs at 30% and 45% mature sizes when fed on pelleted lucerne and pelleted grass as a choice is presented. The relationship at 30% mature size was linear (R^2=0.97, $p<0.001$), such that: Y_{DMI}= 122 (SE±54.3) + 0.790 (SE±0.0473) X_{DMI}, where Y_{DMI} and X_{DMI} is estimated and observed dry matter intake of lucerne; the r. s. d. was 87.0 g/day. However, at 30% mature size the n-alkane technique tended to underestimate the amount of lucerne consumed by a few lambs, particularly at high intake levels. The discrepancy between the observed and estimated amounts was also reflected in the significances of the slope and the intercept from 1 and zero, respectively, of the regression line describing the relationship between the observed and the estimated quantities (Table 3). At 45% mature size there was also a linear relationship (R^2=0.97, $p<0.001$) between the estimated and the observed amounts of lucerne in the selected diet and this relationship was described by the regression equation that: Y_{DMI}= 85.0 (SE±94.4) + 0.911 (SE±0.0521) X_{DMI}, where Y_{DMI} is the estimated dry matter intake of lucerne and X_{DMI} is the observed dry matter intake of lucerne with r. s. d. of 117 g/day.

Figure 6 shows the comparison of the results of using different alkane combinations and recovery rates from Mayes et al. (1986) and Dillon (1993) cited by Hameleers and Mayes (1998) in the least squares calculation for predicting the proportion of lucerne in the selected diets of lambs offered pelleted lucerne and pelleted grass as a choice. The combination of alkanes C_{31} and C_{33} used with the recovery rates from Mayes et al. (1986) gave the closest predictions of the proportion of lucerne in the diet when compared with predictions based on other sets of alkanes and recovery rates. The same combination of alkanes but used with the recovery rates published by published by Dillon (1993) and cited by Mayes and Hameleers (1998) yielded reasonably close diet selection estimates. The combination of C_{33}, C_{31} and C_{27} and that of C_{33} and C_{27} used with the recovery rates of Mayes et al. (1986) also gave reasonably close estimates. Using the set of alkanes C_{31}, C_{29} and C_{27} with the recovery rates of Mayes et al. (1986) yielded the worst estimates of the proportion of lucerne in the diet of the lambs given lucerne and grass as a choice, of the all the combinations of alkanes and recovery rates investigated.

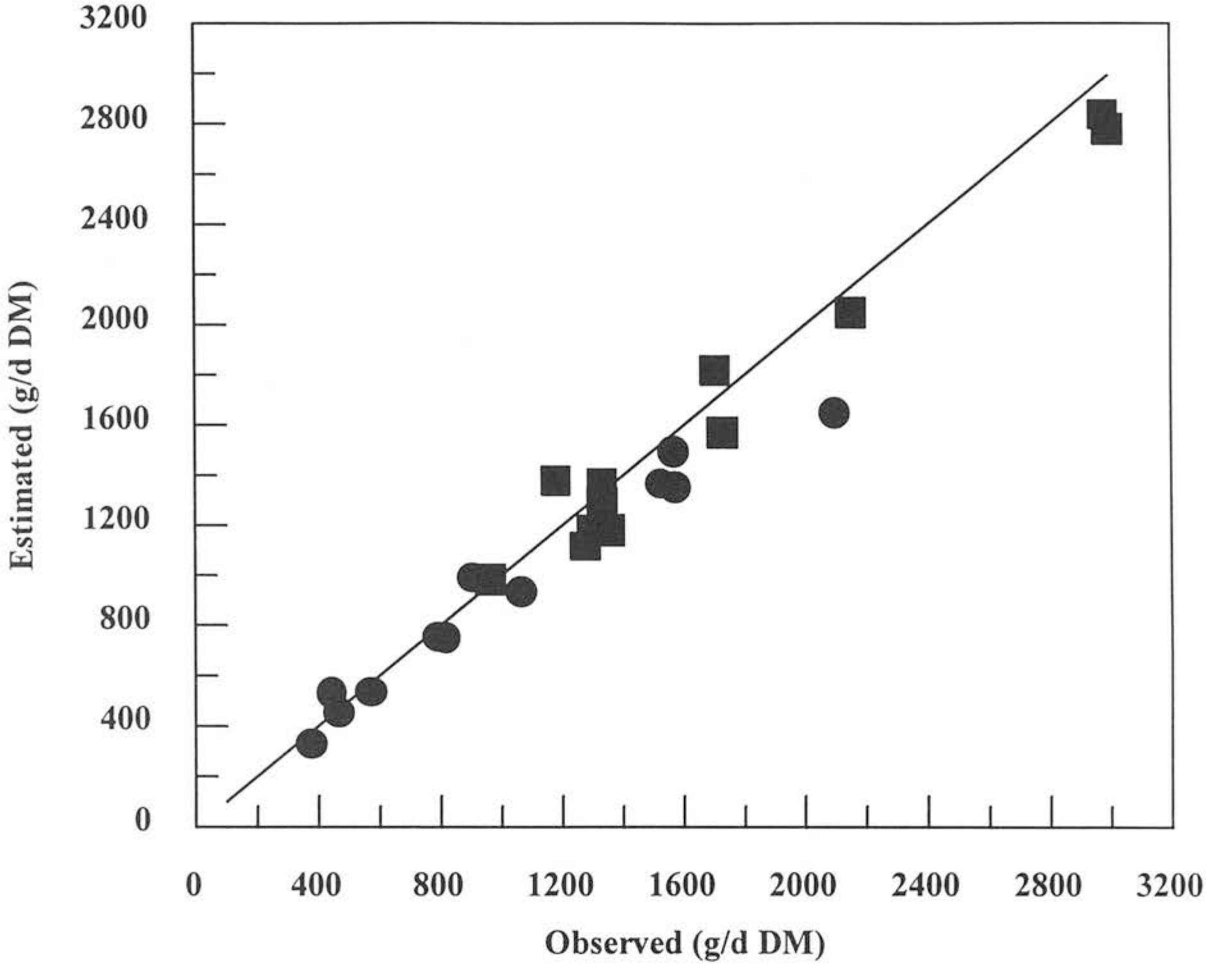

Figure 5 The relationship between the observed and the estimated amount of lucerne in the diet selected by each of the lambs given pelleted lucerne and pelleted grass as a choice at 30% and 45% mature sizes *(n=12)*. ●, 30% mature size; ■, 45% mature size and the bold line indicates the line of equality.

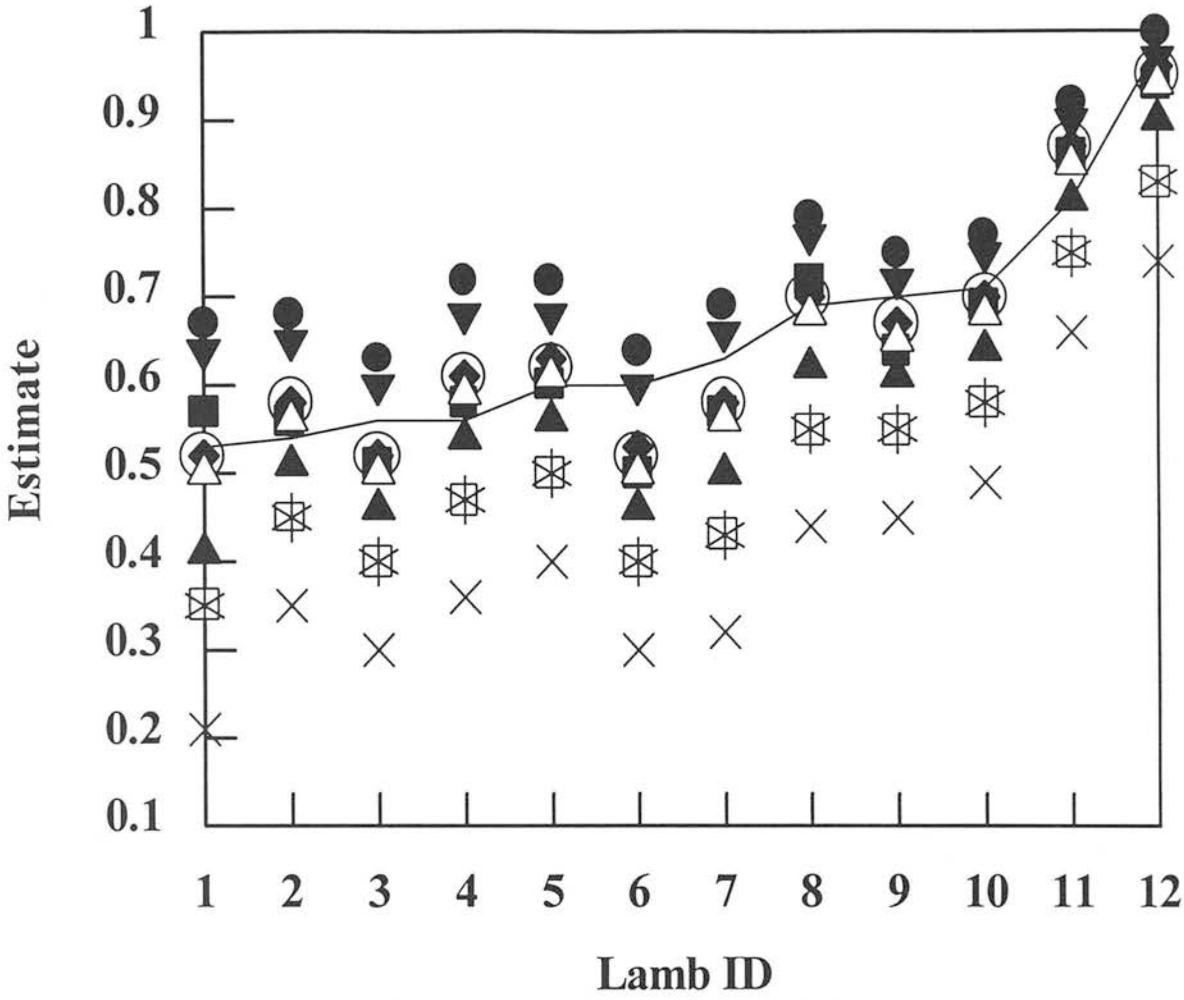

Figure 6. Comparison of the results of using different alkane combinations and recovery rates from Mayes et al. (1986) and Dillon (1993) cited by Hameleers and Mayes (1998) in the least squares calculation for predicting the proportion of lucerne in the selected diets of lambs offered pelleted lucerne and pelleted grass as a choice. The alkanes used and the source of recovery rates are indicated in parenthesis.
—, Observed; O, (Mayes C_{33},C_{31}); ●, (Mayes C_{33},C_{29}); ■, (Mayes C_{33},C_{27}); ,(Dillon C_{31},C_{33}); ▲, (Dillon C_{33},C_{31},C_{29},C_{27}); ✶, (Mayes C_{33},C_{31},C_{29},C_{27}); ▼, (Mayes C_{33},C_{29},C_{27}); □, (Mayes C_{33},C_{31},C_{29}); ◆, (Mayes C_{33},C_{31},C_{27}); **X**, (Mayes C_{31},C_{29},C_{27}).

Experiment 2

The means of the ratios of the concentrations of C_{33}:C_{32} in the faeces of the restricted and *ad libitum* fed lambs over the 24 h sampling period are listed in Table 4. The ratio of the concentration of C_{33}:C_{32} in the faeces was not significantly affected by sampling time over the 24 h sampling period irrespective of whether the lambs were restricted or *ad libitum* fed.

Table 4 The means of the ratio of the concentration of C_{33}:C_{32} in the faeces of restricted and *ad libitum* fed lambs (*n=6*) over a 24 h sampling period.

Feeding	Time						s.e.d.[1]
	08:00	12:00	16:00	20:00	24:00	04:00	
Ad libitum	0.595	0.629	0.631	0.748	0.834	0.763	
							0.0884
Restricted	0.278	0.247	0.289	0.276	0.186	0.252	

[1]s.e.d. for comparison of the ratios of the concentration of C_{33}:C_{32} in the faeces of the lambs at different sampling times within each feeding regime.

Experiment 3

A comparison of the ratio of the concentration of C_{33}:C_{32} in the faeces of once and twice daily dosed lambs over the 5 sampling days are given in Table 5. There was on average a significantly higher *($p<0.05$)* ratio of the concentration of C_{33}:C_{32} in the faeces of the lambs dosed twice daily than in the faeces of those dosed once daily over all the sampling days. However, the ratios for days 1, 2, 3 and 4 were not significantly different between the two dosing strategies; only the day 5 ratio was significantly higher in the faeces of the lambs that were dosed twice daily.

Table 5 The means of the ratio of the concentration of C_{33}:C_{32} in the faeces of once and twice daily dosed lambs ($n=6$) over 5 sampling days. Means with different superscripts within the same row are significantly different *($p<0.05$)*.

	Number of Daily Dosings	
Sampling Day	Once	Twice
First	0.591^{a}	0.721^{a}
Second	0.673^{a}	0.742^{a}
Third	0.469^{a}	0.630^{a}
Fourth	0.570^{a}	0.722^{a}
Fifth	0.457^{a}	0.651^{b}
Mean	0.552	0.693
s.e.d.[1]	0.0813	

[1]s.e.d. for comparison of the means of the ratios of the concentration of C_{33}:C_{32} in the faeces of once- and twice daily dosed lambs.

Discussion

Experiment 1

The close relationship between the observed and the estimated grass dry matter intake (Table 2 and Table 3) found in this study is consistent with the findings of similar studies that investigated the reliability of the n-alkanes method in estimating herbage dry matter intake by ruminants. Mayes et al. (1986) assessed the accuracy of pairing each of two dosed n-alkanes, octacosane (C_{28}) and dotriacontane (C_{32}) with each of their two most adjacent natural n-alkanes to estimate the intake of perennial ryegrass by sheep. All the pairing methods used slightly underestimated actual intake, except the C_{32} dosed-C_{33} natural alkane pair, which gave an estimate that equalled the measured intake. The experiment reported here also made use of the C_{32} dosed-C_{33} natural alkane pair to estimate intake, but the pair gave results which, on average, slightly overestimated intake at 30% mature size. The alkane technique however underestimated by 350g the dry matter intake of grass by one of the lambs at 30% mature size. It is difficult to offer an explanation for these discrepancies, particularly in the light of the close agreement between the rest of the observed and estimated quantities (Table 3), except in terms of experimental error, to which both the estimated and measured quantities are subject. Newman et al. (1998) pointed out that there is a possibility of analytical error in the determination of alkane concentrations in the diet and in the faeces, which could have a significant effect on the estimates derived by the alkane method. Error can also be introduced by a failure to obtain a representative sample from the consumed diet (Dove and Mayes, 1991;

Newman et al., 1998). However this is not a likely source of error in this study as the consumed diet had been ground and pelleted, which should have resulted in a fairly homogeneous mixture of the various components (parts from the same plants and from other plants) making up the pellets. The observed intake amounts, although to a much lesser degree than the estimated amounts, are also subject to a small degree of measurement error, which could influence the agreement between the observed and estimated values. At 45% mature size however the technique gave estimates which were in close agreement with the measured intake (Figure 1); the fitted regression line describing the linear relationship was not significantly different from the line of equality [i.e. the slope and the intercept were not significantly different from one and zero, respectively (Table 3)].

As shown in Figure 2 and in Table 3, the dry matter intake of lucerne was accurately estimated at both levels of maturity. At both 30% and 45% mature sizes there was a linear relationship between the observed and the estimated lucerne dry matter intake and the fitted regression line describing the linear relationship was not significantly different from the line of equality (Table 3). The results are consistent with those reported by Vulich et al. (1991). They reported a bias, expressed as estimated intake minus observed intake as a percentage of observed intake, of 3% when using the same pair of alkanes (C_{32} dosed-C_{33} natural) as in this study to estimate herbage intake by sheep. The mean biases found in this study were 5.2% and 1.4% for the 30% and 45% mature size groups, respectively.

The success of the n-alkanes method to estimate diet composition depends on the accuracy of the procedure used to derive the best match between the concentrations of certain n-alkanes in the constituents of the diets on offer and the concentrations of the same n-alkanes in the faeces, after correcting for losses in the digestive tract. Several mathematical methods have been suggested, the earliest of which made use of simultaneous equations, as described by Dove and Mayes (1991) and Dove (1992). In order for the equations to be solvable, the number of n-alkanes whose concentrations are used in the equation should ideally be equal to the number of the individual components of the diet, although a solution can be obtained by using one alkane less than the number of diet components, as described by Dove and Mayes (1991). However when this method is used to resolve a diet with only two components, in which case the concentrations of two n-alkanes should ideally be chosen and used in the simultaneous equations, it is not always obvious which two to chose from several possibilities. The solutions may differ, depending on which two alkane concentrations are used in the two simultaneous equations (Newman et al., 1995).

Recently, least squares optimisation and iterative methods, which allow the use of more alkanes than the number of components making up a diet, have emerged as the most appropriate ones because they yield unique diet proportion estimates from the concentrations of several n-alkanes in the faeces and the diets on offer. Hameleers and Mayes (1998) compared the performances of three such methods in estimating the composition of the diet selected by dairy cows offered a perennial ryegrass and white clover mixture: the iterative optimisation procedure described by Mayes et al.

(1994), the non-negative least squares iterative method described by Dove and Moore (1995) and the non-negative least squares method using matrix algebra proposed by Newman et al. (1995). All the three methods yielded similar estimates of the proportion of white clover in the diet of the cows, which suggested comparable degrees of accuracy in predicting diet selection.

The technique used in this study, which is similar to that described by Newman et al. (1995), accurately estimated the proportion of lucerne in the diet selected by the lambs when given lucerne and grass as a choice (Figure 3). In addition, the proportion estimates derived and used in the Mayes et al. (1986) formula to estimate DMI accurately estimated DMI of grass at both levels of maturity (Figure 4 and Table 3). The DMI of lucerne was also adequately estimated at both stages of maturity, particularly at 45% (Figure 5 and Table 3). However, unlike in the results of Hameleers and Mayes (1998), where white clover was slightly overestimated, lucerne at 30% mature size was slightly underestimated in this study. Any one or more of several sources of error could have caused the discrepancies between the estimated and the measured values. For instance, Newman et al. (1998) cautioned that errors in the extraction and quantification procedures may yield incorrect alkane concentrations of the diet components, which may in turn affect the diet proportion estimates derived from the technique. These concerns did not seem to play a major role in this study because the estimated quantities were in good agreement with the measured, except for the lucerne underestimation at 30% mature size. Even at this stage of maturity there was a linear relationship between the observed and the estimated amount of lucerne in the selected diet (R^2=0.97, $p<0.001$), which lends credence to the alkane technique. However, the DMI of lucerne by one lamb was underestimated by almost 450 g, and this seems unlikely as none of the other lambs had observed lucerne DMI figures of the order suggested by the measured intake amount for this lamb. As has previously been mentioned, the measured intake amount may not be free of experimental error.

The close relationship between the estimated and the measured DMI quantities show that the technique can be successfully used to estimate the composition and amounts of individual components of the diet selected by animals offered lucerne and ryegrass as a choice, at least under controlled conditions. These results are also consistent with the findings reported by other researchers who used essentially the same technique used in this study to estimate herbage intake and selection by ruminants offered a choice of diets (Hameleers and Mayes, 1998; Salt et al. 1994).

In this study the concentrations of C_{31} and C_{33} (recovery rates from Mayes et al. (1986)) were used in the least squares calculations because they gave the closest predictions of the proportion of lucerne when compared with predictions based on other sets of alkanes (Figure 6). In instances where there are no observed quantities for validation, it may not be as obvious which sets of alkanes to use in order to establish the best diet proportion estimates. Under such circumstances the sensible approach would be to opt for a method which derives diet proportion estimates from as many of the available alkanes as possible, unless, as was the case in this study, there is reason to chose a method that constructs estimates from fewer alkanes.

Regardless of the method used, the best estimates are established when the alkane concentration patterns are markedly different and the total alkane contents are similar between the diets on offer (Dove and Mayes, 1991).

However, in this study certain alkanes tended to have a greater influence on the accuracy of the diet proportion estimates than others. For instance exploring the consequences of using another combination of two alkanes besides the two, C_{31} and C_{33}, used to derive the estimates used in this study produced unexpected results. Substituting C_{29} for C_{31} in the equations did not yield diet proportion estimates that are similar to those yielded when using C_{31}, yet the difference margins in the concentrations of these alkanes between the two diets are comparable. Substituting C_{27} for C_{31} gave similar estimates to the ones obtained when C_{31} was used, yet the concentrations of C_{27} in lucerne and ryegrass were similar. This was unexpected because the sensitivity of the estimates produced by least squares methods or simultaneous equations is expected to improve as the difference in alkane concentration between the two component diets increased. Leaving out C_{33} and using any other pair yielded estimates which were different from the observed proportions of lucerne in the diet, which implied that of all the four alkanes which could be used C_{33} had the most influence on the accuracy of the estimates produced. Thus the individual alkanes could be ranked in order of influence on the estimates of lucerne proportion in the selected diets as C_{33}, C_{31}, C_{27} and C_{29}. A possible explanation for this difference is that the actual recovery rate of lucerne C_{29} may be different from the one assumed in this experiment and the recovery rates of the others were close to those assumed here. Newman et al. (1998) cautioned that incorrect recoveries may have a large and significant effect on the estimates derived from least squares optimisation techniques. This is also evident when examining the difference between the estimates generated using the recovery rates use by Mayes et al. (1986) and those used by Dillon (1993) as reported by Hameleers and Mayes (1998) (Figure 6) The two sets of recovery rates were established under different conditions and using different animal species. Dillon and Stakelum (1990) reported lower recovery rates than found previously with lactating dairy cows. Stakelum and Dillon (1990) found a reduction in the recovery rates of C_{31} and C_{32} in dairy cows at higher DMI levels. Mayes et al. (1986) found no evidence of the influence of feeding level on the recovery of alkanes in sheep fed mainly on ryegrass and a concentrate supplement. However, the recovery of C_{27} was lower when the supplement was given and when a daily dose of C_{28} and C_{32} was mixed with palmitic and stearic acids. Therefore the behaviour of alkanes in the digestive tract may be influenced by animal species and type, the level of feeding and diet and these could affect faecal recovery rates, which could in turn affect diet proportion estimates generated by the alkane technique.

Experiment 2

There was no evidence of within day variation in the ratio of the concentrations of C_{33}:C_{32} when the lambs were restricted or *ad libitum* fed. These results are consistent with those reported by Mayes et al. (1986), who found no evidence of diurnal variation or feeding level effect in the ratios of concentrations of natural to dosed alkanes in the faeces of lambs. Similarly, Malossini et al. (1994) found low diurnal variations in the ratio of the concentrations of natural to dosed alkanes in the faeces of grazing dairy cows. Thus, for this study, unbiased intake estimation can be made using faecal samples collected any time of the day after a steady state had been established. Although Stakelum and Dillon (1990) reported diurnal variation, they found no evidence of feeding level effect in the excretion pattern of the ratios of natural to dosed alkanes by dairy cow.

Experiment 3

For accurate DMI estimation by the alkane method the concentration of the dosed alkane in the faeces should be constant. To ensure this constancy it is suggested the dose is administered twice daily (McMeniman, 1997). However, dosing once daily would reduce animal handling and would thus be more practical, particularly under extensive grazing conditions. Dillion and Stakelum (1990) reported a greater within day variation with once daily than twice daily dosing in the ratios of natural to dosed in the faeces of dairy cows. The results of this experiment suggested no difference in the ratios of the concentrations of C_{33}:C_{32} between the two dosing strategies until the fifth day of dosing (Table 4). The possible cause of this variation may have been that all the six lambs that were dosed twice daily were fed on grass and three of the six lambs that were dosed once daily were unwittingly fed on lucerne which has a lower concentration of C_{33} than grass. That there were three lambs fed on lucerne in the group that was dosed once daily could have reduced the concentration of C_{33} in the faeces of these lambs, which could have confounded the difference in the concentration of C_{33} due to the difference in dosing strategies between the two groups of lambs. Feeding the same diet to all the lambs in both groups would have provided a fairer basis for comparing the two dosing strategies. There was however no evidence of day to day variation in the ratios of the concentrations of C_{33}:C_{32} associated with either strategy.

Conclusion

The potential of the alkane technique in estimating the DMI and diet of stall-fed or outdoor ruminants has been demonstrated (e.g. Hameleers and Mayes, 1998 and Salt et al., 1994). Vulich et al. (1991) found it not only accurate and precise, but also repeatable. The close agreement between the estimated and observed parameters investigated shown by the results of this study also attest to its accuracy The close agreement between the measured and observed parameters at both lamb mature sizes demonstrated that the reliability of the technique was not affected by how much food the lambs ate. However, improving certain aspects associated with the technique would enhance its accuracy. For instance, in this study, if there were no direct intake measurements against which to compare estimates, it would not have been obvious which two alkanes to use in the least squares optimisation part of the procedure and using the wrong ones could have yielded incorrect estimates (Figure 6). The possible reason for the difference in generated estimates when different alkanes are used may be that some or all of the recovery rates assumed were not quite appropriate for this study.

Recovery rate errors have far-reaching consequences because they are used in the primary computation for estimating the proportions of individual components of a mixed diet, upon which the subsequent total DMI and partitioning into amounts of the individual components of the total diet are based. Therefore if the recovery rates are incorrectly determined, the estimates of the DMI of the individual components of a mixed foodstuff will carry these errors in addition to other errors that may creep into the other steps involved in the procedure. Recovery rates determined under similar experimental conditions would yield similar results regardless of the alkanes used, provided the quantities of the chosen alkanes were similar, but patterns different between the two diets on offer. It can therefore only improve the ability of the alkane technique to estimate diet selection to have a body of recovery rates that have been established with different animals when fed different types of diets at different levels of feeding. Alternatively and more appropriately, for each experiment, recovery rates for each alkane in each diet should be determined experimentally before the diet selection study starts and these recovery rates, rather than the published ones, should be used in the calculations.

The results of experiment 2 did not show evidence of within day variation in the ratios of the concentrations of C_{33}:C_{32} when the lambs were either restricted or *ad libitum* fed once a steady state has been established. A conclusion that can be drawn from such results is that in order to make unbiased intake estimation faecal sample collection should not be limited to specific times of the day, which lends support to the simplicity and minimal evasiveness attributes of the technique.

It would be difficult to draw meaningful conclusions from the results of experiment 3 because, while all the lambs that were dosed twice daily were fed on lucerne, one half of those that were once daily dosed were fed on lucerne and the other half were inadvertently fed on grass. Therefore variation in the ratios of the concentrations of

C_{33}:C_{32} between the two dosing strategies will be confounded by the difference in the concentration of C_{32} and C_{33} between grass and lucerne, which would rule out a fair comparison between the two dosing strategies. A fairer investigation into the difference in the ratios of C_{33}:C_{32} between the two dosing strategies is thus warranted in order to establish whether or not once daily dosing is adequate for accurately determining DMI by animals.

CHAPTER 4

Introduction

Voluntary intake of tropical pastures by ruminants is generally low compared with that of temperate pastures, but varies according to sward type and time of the year. Most tropical pasture types grow rapidly during the rainy season, which leads to their maturity and lignification by the time the dry season comes (Preston and Leng, 1987). During the dry season the available forage is of low digestibility and quality and its intake by animals is not enough to maintain good animal performance (Preston and Leng, 1987). The response of animals to seasonal fluctuations in food quality varies from animal to animal according to genetic potential. In order to formulate efficient supplementary feeding it would be useful to know intake potentials of individual animals. Mayes et al. (1986) proposed a double marker method, which uses a synthetic even-chain and a natural odd-chain alkane, for calculating dry matter intake of individual grazing animals. Various investigations have compared the accuracies of different pairs of alkanes and the C_{33} and C_{32} pair has been found to give the most accurate estimates of the dry matter intake of temperate forages (Mayes et al., 1986; Vulich et al., 1991; Hameleers and Mayes, 1998). However, forages have different alkane profiles and the amount of C_{33} they contain may not be enough to allow its use (Laredo et al., 1991 and Hameleers and Mayes, 1998). Other alkane pairs can also be used, but accuracy may be compromised if there is a large difference in recovery rates between the two alkanes (Dove and Mayes, 1991). The alkane C_{32} (to pair with C_{33}) was not available during the time this experiment was conducted and this necessitated the use of C_{36} which was also cheaper than C_{32}. Because the recovery rate of C_{36} is closer to that of C_{35} than to that of C_{33} (Dove and Mayes, 1991), C_{35} was chosen as the natural alkane to pair with C_{36}.

The aim of this experiment was to use the alkane pair of C_{36} and C_{35} to compare the voluntary dry matter intake of *Brachiaria decumbens* by dry and lactating cows between the dry and wet seasons of Bolivia, South America.

Material and methods

The experiment was conducted during two seasons in 1997, towards the end of the rainy season in early April and in the middle of the dry season in mid July. During the rainy season eight Criollo dry cows averaging 301 kg liveweight and eight lactating cows in mid lactation, averaging 5 l of milk per day, and averaging 320 kg liveweight continuously grazed for five days each of three 7.5 ha *Brachiaria decumbens* paddocks. During the dry season eight lactating averaging 300 kg liveweight and seven dry cows averaging 289 kg liveweight continuously grazed for 7 days in each of two 11 ha *Brachiaria decumbens* paddocks. Each paddock had herbage mass content of 7-8 ton of DM/ha and therefore at no stage was herbage mass availability limiting intake. The cows were on the paddocks throughout the day and night and were removed only for milking twice a day in the morning and afternoon and were normally returned within an hour of removal.

Each cow was dosed over a 12-day period twice daily at 06:00 and 15:00 with 400 mg (2x200mg) of hexatriacontane (C_{36}) impregnated into a cellulocotton filter. From day eight faecal grab samples were collected from each cow twice daily at dosing time (06:00 and 15:00) for five days. The samples were oven dried at 60^0 C for 48 hours and then ground. For each cow, the two faecal samples from each day were bulked into a daily sample and an aliquot extracted as described by Mayes et al. (1986), with the modifications that duplicate 0.5g samples were directly heated in ethanolic potassium hydroxide and 7ml of heptane were used as the solvent.

Hand-plucked samples collected from each of the paddocks on the first grazing day during each season were oven dried at 60^0 C, milled and pooled to make a composite sample of the paddocks grazed in each season. An aliquot (1 g) was extracted in duplicate the same way as faeces, except that about 50% more solvent and water than in faecal extraction were used. The final eluates were injected onto a capillary column (HP.5 Crosslinked Phenyl Methyl Silicone, 50m x 0.32mm x 0.17μm) in a model HP6890 gas chromatograph fitted with a flame ionization detector and linked to a PC run by an HP Chemstation software. The oven temperature programme was initially 65^0C and then rose at 10^0C/min to 300^0C where it was held for 20 min. The temperatures of the injection and detector ports were 325^0C and 350^0C, respectively. The flow rate of the carrier gas (N_2) was 33.4ml/min. and that of the makeup gas (N_2) was 24ml/min. The ratio of the peak areas of the analysed n-alkanes to that of the internal standard (C_{34}) was used to calculate n-alkane amounts in the samples. Identification of the different n-alkanes was made based on the relative retention times of known standards.

Several filters from the batch that was used for dosing the cows were extracted to determine the average amount that was adsorbed onto each filter. The method involved slicing three pellets and boiling them for 1h under reflux in 100ml of heptane after adding 100mg of internal standard (C_{34}), (R. W. Mayes unpublished protocol). After partial cooling to about 60^0C an aliquot was taken and analysed by gas chromatography the same way as the faecal and feed samples.

The amount of dry matter eaten by each cow was estimated from the concentrations of C_{35} and C_{36} n-alkanes in the faeces and the consumed herbage in each season according to the method described by Mayes et al. (1986). The intake estimate data were analysed as a completely random design by Genstat 5 (Lawes Agricultural Trust, 1998) using a two way ANOVA.

Table 1. The average concentrations of C_{35} and C_{36} (mg/g DM) in hand-plucked herbages in the paddocks grazed in the dry and rainy seasons.

Season	C_{35}	C_{36}
Rainy	0.0227	Not detectable
Dry	0.0638	Not detectable

Results

The concentrations of C_{35} and C_{36} in the herbage, which was predominantly *Brachiaria decumbens*, grazed by the cows in the dry and the wet seasons are presented in Table 1. There was almost three times as much C_{35} in the herbage during the dry season as there was during the wet season. The concentration of C_{35} in the wet season was slightly less than that detected by Dove and Mayes (1991) in *Brachiaria decumbens*. During both seasons the concentration of C_{36} was too low to detect and for the purposes of the intake estimation calculations it was assumed to be zero.

In Table 2 the comparison of the dry matter intake amounts of the cows between the dry and the wet seasons and between the lactating and the non-lactating stages of production are shown. During the rainy season the lactating and the non-lactating cows consumed significantly more ($p<0.05$) dry matter than during the dry season. The average dry matter intake of the lactating cows was about 1 kg more ($p<0.001$) than that of the non-lactating cows when intake was combined for both seasons.

Table 2. The comparison of the voluntary dry matter intake (kg/d) of the cows between the dry and rainy seasons and between the lactating and nonlactating stages of production.

Season	Production Stage		Means	SED
	Lactating	Nonlactating		
Rainy	7.6 8.1 7.6 7.5 7.3 7.8 7.7 7.7	5.9 6.7 7.3 5.9 6.4 7.8 6.5 6.2	7.1 (n=16)	0.24
Dry	7.4 5.2 6.6 6.7 7.4 7.1 6.3 7.2	5.4 4.6 5.6 5.5 7.0 5.7 7.0	6.3 (n=15)	
Means	7.2 (n=16)	6.2 (n=15)		
SED	0.24			

Discussion

Voluntary intake of most tropical pastures by ruminants is estimated to vary between 1% and 3% of body weight per day, depending on sward type and season of the year (Preston and Leng, 1987). The average daily dry matter intake estimates of 7.1 kg during the rainy season and 6.3 kg during the dry season are well within this range (Table 2). Reported voluntary intake of *Brachiaria decumbens* by cattle and sheep has been variable, but there are no published data obtained by using the alkane technique. In Brazil the daily intake of *Brachiaria decumbens* by steers was found to be 45 and 70 g DM/kg $LW^{0.75}$ for mature and immature stands, respectively (Lascano and Euclides, 1996). These figures are lower than the intake estimates obtained during the dry and wet seasons in the study reported here. However the seasonal difference, but not the amount, is in agreement with the higher rainy season and lower rainy season estimates obtained in this study. Grieve and Osbourn (1965) cited by (Lascano and Euclides, 1996) reported an intake of young *Brachiaria decumbens* re-growth of 80 g DM/kg $LW^{0.75}$ by Jamaican sheep. In contrast, in Brazil the intake by sheep was considerably lower when the same grass type was fed as hay from forage harvested every six weeks (Lascano and Euclides, 1996)

The higher intake estimates for the lactating cows in both seasons are in agreement with the general belief that lactating cows increase their intake in order to meet the higher nutrient requirements of lactation (Forbes, 1993; Weston, 1996). Food availability and quality, however, often prevent intake meeting these requirements (Weston, 1996). *Brachiaria decumbens,* like all tropical grasses, decreases in digestibility as it matures from the rainy to the dry seasons, which reduces its intake by livestock (Humphreys, 1991). However, it shows a lower rate of decline in nutritive value and digestibility than grasses in other genera, which explains lower intake of other grasses by ruminants during the dry season and sometimes during the wet season (Humphreys, 1991). Euclides et al. (1993) compared the green dry matter (GDM) and leaf dry matter (LDM) available over the growing season between *B. decumbens* and *B.brizantha. B. decumbens* had a higher leaf dry matter than *B. brizantha* at the end of the dry season. However, *B. brizantha* had a higher GDM in the rainy season. This difference in LDM and GDM between the two species during the rainy and the dry season was reflected in the average daily gains of steers grazing them. Steers grazing *B. brizantha* showed higher average daily gains during the rainy season, but lower average daily gains during the dry season than those grazing *B. decumbens*. Similarly, Cardoso et al. (1993) found *B.decumbens* capable of fattening Nellore steers during the rainy season in Brazil and also maintained their liveweight during the dry season at a stocking rate of 1 head/ha.

Voluntary intake of another related species, *Brachiaria humidicola*, during the rainy season in Colombia was lower at 1.3 per cent of the bodyweight of cattle (Lascano and Euclides, 1996) than the intake estimates obtained in this study. This species normally has a lower crude protein content than *B. decumbens* (6% vs. 14%), hence its lower intake. At the same stocking rate, this species was also reported to produce 115 kg/ha less annual average daily gains than *B. decumbens.*

The alkane technique has been successfully used to estimate the intake of different forages by sheep and cattle. For temperate forages estimates with the least bias were obtained when the pair of the dosed alkane C_{32} and the natural alkane C_{33} were used. For instance, Stakelum and Dillon (1990) compared the herbage intake estimates of dairy cows, generated by using various alkane pairs and C_{35} as an internal marker together with faecal output. Their results suggested that the best estimates were obtained by using the alkane pair of C_{32} and C_{33}, and the poorest estimators were the pair of C_{35} and C_{36} and the C_{35} and internal marker method. Mayes et al. (1986) did not include the pair of C_{35} and C_{36} in an investigation comparing different alkane pairs and C_{35}, used as an internal marker, plus faecal DM output to estimate the intake of perennial ryegrass or ryegrass and a concentrate diet. The pair of C_{33} and C_{32} gave identical intake estimates to the actual intakes of the lambs. They concluded that other alkane pairs, although slightly underestimated intake, gave reasonably estimates.

Dove et al. (1990) compared the intake estimates of perennial ryegrass by sheep over several stages of lactation, calculated using in vitro-based digestibility and digestibility derived from herbage and faecal concentrations of C_{35}. From the consistency of the C_{35} alkane-based results of the study and in relation to published intake figures, they concluded that the alkane-based were more accurate than the in vitro-based estimates. Unal and Garnsworthy (1999) compared the intake estimates derived by using the pairs of C_{32} and C_{33} and C_{36} and C_{33} against the observed intake of dairy cows fed on a mixture of silage, concentrates and sugar beet pulp nuts.

Slightly better intake estimates were obtained when C_{32} was used as the dosing alkane; intake was underestimated by 0.5 kg when C_{36} was used as the dosing alkane.

Laredo et al. (1991) explored the potential for using alkanes in tropical forages, including *Brachiaria decumbens*, as markers for the determination of dry matter by grazing ruminants. They concluded that where the forage concentrations of C_{35} are higher than 50 mg/kg dry matter the pair of C_{35} and C_{36} could be used to used to estimate dry matter intake. However, with forages that contain enough concentration of C_{33}, this alkane could be paired with dosed C_{32} to obtain low error intake estimates. For forages that contain insufficient amounts of C_{33} a shorter chain length alkane could also be used, but with a reduction in the accuracy of the intake estimates.

The results of the present study have not been validated by concurrent direct intake measurement, but the estimates obtained using the pair of C_{35} and C_{36} should be reasonably accurate. The two alkanes have comparable recovery rates (Dove and Mayes, 1991), a requirement for accurate intake estimates (Mayes et al., 1986; Laredo et al., 1991; Dove and Mayes, 1991). Casson et al. (1990) cited by Laredo et al. (1991) recommended that the concentration of the odd chain alkane used in the method be more than 50 mg/kg of herbage DM. However, they added that this depends on the degree of accuracy of which laboratories are capable and also the amount of precision required. The concentration of C_{35} in the herbage grazed during the wet season in this study was less than this suggested threshold (Table 1), but the unavailability of C_{32} (to pair with C_{33}) during the time the experiment was conducted necessitated the use of C_{36} for dosing. Since the recovery rate of C_{36} is closer to that of C_{35} than to that of C_{33} (Dove and Mayes, 1991), C_{35} was chosen as the natural alkane to pair with C_{36}. That the concentration of C_{35} in the wet season herbage was less than the threshold of 50mg/g DM was probably not a major source of error in the results of this study. This laboratory used herbage which had a lower alkane concentration (lucerne C_{33} = 17.4 mg/kg DM) than this threshold and obtained intake estimates which closely agreed with the observed intake of sheep (see results of experiment 1 in Chapter 3 of this manuscript).

In addition, the results of an experiment by Dove et al., (1990) showed that C_{35} in perennial ryegrass can be used to obtain more accurate digestibility estimates than an *in vitro* procedure. Although, these authors did not report the alkane profile of the ryegrass used in their study, it normally contains low concentrations of C_{35} (Hameleers and Mayes, 1998b; Dove and Mayes, 1991) and the low concentration did not seem to be a source of considerable error in the study.

Conclusion

In cases where C_{32} is not available C_{36} could be used as the dosed alkane in conjunction with a natural alkane to estimate the dry matter of intake of tropical forages. The choice of which alkane to pair it with should be based on its similarity to the recovery rate of C_{36} rather than the amount available, as long as the concentrations are detectable. If the closest natural alkanes to C_{36} are not detectable, then lower natural alkanes can be used, but the degree of accuracy of the estimate may be compromised. Dove and Mayes (1991) contend that for every percentage point difference between the recovery rates of the dosed and the natural alkanes, the intake estimates derived will be one percentage point inaccurate. As long as the disparities between the actual and the estimated intake values are small and similar during both seasons, the method will have achieved the aim of this study- to compare voluntary intake of *Brachiaria decumbens* by cows during the wet and the dry season. The degree of accuracy may still be better than most other available methods for estimating the intake of grazing animals can achieve (Dove et al., 1990). It would however be useful to carry out more studies using the alkane pair of C_{35} and C_{36} to estimate dry matter intake of tropical pastures by cows.

CHAPTER 5

Introduction

The amounts of poor quality forages eaten by ruminants even under *ad libitum* feeding conditions are often insufficient to support the level of production of which animals are capable (Forbes, 1993). This low intake may be a result of restricted flow of forage digesta through the gastrointestinal tract, which may cause distension of one or more segments of the gut (Allen, 1996). In such circumstances the amount of dry matter consumed by a ruminant during a meal is influenced by the rate of clearance from the rumen of previously ingested food (Forbes, 1986). Rumen clearance occurs by solubilization of soluble material, degradation of degradable material and passage of the less digestible residues from the reticulorumen into the omasum (Van Soest et al., 1988; Kennedy and Doyle, 1993)

The rate of passage of particles from the rumen depends on the efficiency of their comminution into small particles, mostly by ruminative chewing, which is influenced by the physical properties of the particles (Murphy and Kennedy, 1993). Reducing digesta particle size not only speeds their rate of passage but it also increases their rate of fermentation in the digestive tract as a result of an increase in surface to mass ratio for microbial attack (Gerson et al., 1988). In contrast, larger particles promote slower passage and do not allow as great a nutrient intake as smaller particles (Ehle and Stern, 1984). The range of particle sizes of forage digesta then determines not only the efficiency at which they are digested, but also the rate at which undigested residues pass along the gut. It has also been proposed that density or buoyancy is another characteristic by which particles are sorted for selective retention in or passage out of the reticulorumen. For instance Kennedy and Murphy (1988) found a close relationship between reticulorumen retention time and specific gravity of feedstuffs of various lengths. However, Kennedy (1995) could not establish a connection between the specific gravity of particles, measured as particle sedimentation rate (Sutherland, 1988), in the reticulum and their probability of passage to the post-ruminal tract of swamp buffaloes. Thus a clear elucidation of the kinetics of digestion of forages of different physical characteristics (e.g. density and size) in the digestive tract, and the physiological factors that modulate them will benefit efforts to predict and enhance the intake of forages by animals.

Plastic particles have been used to provide information about selective retention of particles of different characteristics (Kaske and Engelhardt, 1990). Inert particles with densities 1.2-1.4 g/ml seem to have the most rapid rates of passage in the gut (Kaske and Engelhardt, 1990; desBordes and Welch 1984; Murphy et al., 1989). However, because plastic particles do not undergo the same changes like hydration and specific gravity increase that occur in feed particles in the gut, data obtained from their use cannot provide absolute rates of feed particle movement (Uden et al., 1980).

Heavy metal ions such as those of chromium (Cr) and rare earth elements have been attached to fibre particles and used as solid digesta markers (Uden et al, 1980). Cr forms plant cell wall and protein complexes that are stable in *in vitro* digestion processes (Uden et al., 1980). Because of the tightness of its attachment, Cr reduces the digestibility and increases the density of the material it is intended to label (Ehle, 1984; Van Soest et al., 1988). Rare earths, although variable in degree of attachment, can reduce digestibility and estimates of passage rates obtained through their use may be influenced by the method of attachment (Mader et al., 1984). Another problem with rare earth metals is that their wide reactivity with proteins and carbohydrates could result in the formation of insoluble complexes likely to move independently of the feed particles they were intended to mark (Van Soest et al., 1988).

Long chain n-alkanes have been used successfully to estimate dry matter intake and digestibility in animals. Their affinity for solid digesta shows a potential for their use as markers for investigating the rate of passage of solid material through the gut (Mayes et al., unpublished data). It is not known whether addition of n-alkanes to forage particles would interfere with the degradation of the forage to which they were attached when used in moderate amounts. The aims of this trial were to investigate (I) the effects of addition of n-alkanes on fermentation characteristics of forage particles, and (II) the rates of passage of n-alkane-coated fibre particles of two sizes and two densities in the gastrointestinal tract of sheep.

Gas production experiment

A gas production study was conducted to investigate the effect of coating feed particles with synthetic *n*-alkanes on feed degradation. Hay samples ground to pass through a 1 mm screen in a hammer mill and coated with 0, 12, 24 and 60 mg of hexatriacontane (C_{36}) were compared for *in vitro* fermentation dynamics as described by Jessop and Herrero (1996). Measurements of gas production were taken at 1, 2, 3, 4, 6, 8 h; thereafter every 4 h until 60 h, and at 72, 78, 84, 96, 108, 120 and 144 h. Cumulative gas volumes were corrected for fermentation of 200 mg and for fermentation of soluble material by subtracting the gas produced in 4 h (Herrero and Jessop, 1996), and fitted to the model $GAS=A+B(1\text{-}exp^{-c(t-lag)})$, where A is the asymptote gas production from the fermentation of soluble fraction (gas produced up to 4 h (cm^3)), B is the asymptote gas production from the fermentation of NDF (cm^3), c is the fractional rate of gas production per hour, and lag is the lag phase before the fermentation of NDF begins (h) (Jessop and Herrero, 1996). An ANOVA using the Minitab statistical package (1993) was performed to compare the degradation parameters of the treatments. An LSD was used to compare significant treatment means.

Rates of passage experiment

Experimental design

Four ruminally cannulated Scottish Blackface wethers were used in a cross-over design. The first period was in early December 1997 and the second period was in early January 1998. The sheep were kept indoors and penned individually on a slatted platform above a concrete floor. Two were fed on pelleted grass *ad libitum* and two were fed on baled hay *ad libitum* for 14 days before pulse dosing and for the duration of each period of the trial.

Dose preparation

Two portions of hay from the same batch from which the sheep were fed during the trial were taken and ground through a hammer mill, one to pass through a 6mm screen and the other to pass through a 1mm screen. The fraction that was ground through a 1 mm screen, which was labelled the small, buoyant pool (SB), was coated with n-octacosane (C_{28}) at a rate of 25 mg per gram of hay. The coating was achieved by dissolving the required amount of n-alkane in heptane heated to 80^0C and soaking the hay in the n-alkane solution while continually stirring for about 15 minutes. The hay was then dried in a fume cupboard for at least 24h and oven-baked at 100^0C for 1h to melt the n-alkanes into the hay.

About 1/4 of the hay ground through a 6mm screen, which was labelled the large, buoyant pool (LB), was coated with n-hexacosane (C_{26}) using the same procedure used to coat SB. The rest of the hay milled to pass through a 6mm screen was incubated for 120h in a mixture of 1 part strained rumen liquor and 3 parts buffer solution (Menke et al., 1979) contained in flasks stoppered with fermentation locks and kept in a waterbath at 39^0C. The flasks were shaken 4-6 times a day. As a general guide, about 800ml of the rumen liquor and buffer solution mixture were enough to incubate 15 grams of grass. At the end of the incubation period the rumen liquor was strained out through 2 layers of muslin and discarded while the hay was collected and oven-dried at 60^0C for 48 hours before it was separated into two roughly equal fractions. One fraction, which was labelled the small, dense pool (SD) was ground to pass through a 1mm screen in a hammer mill and then coated with n-dotriacontane (C_{32}); the other, which was labelled the large, dense pool (LD) was labelled with n-triacontane (C_{30}) without grinding. Both fractions were then coated with n-alkanes following the same procedure used for the unfermented hay.

Dosing and sampling

Five grams (coated with 125 mg of n-alkane) of each of the four particle pools were weighed into tissue paper and inserted into the rumen of each of the four sheep via the rumen cannula. Faecal samples were collected from the floor: the first one was collected before or shortly after dosing and thereafter at 3-to 5-hourly intervals until 84h and then at 6-to 9-hourly intervals until 152h. During each collection period, the floor area directly underneath each animal pen was swept clean and the first sample to be voided by each animal was collected and its time recorded. The timing of the faecal collection was aimed at obtaining a sample from each animal as close as possible to 4-hourly intervals in the first 84h and thereafter as close as possible to 8-hourly intervals for the remainder of the collection period. However faecal samples were often voided 1 to 2h before or after the targeted time.

Extraction and gas chromatographic analysis

Faecal samples were oven dried at 60^0 C for 48h, ground and then bagged separately in a resealable plastic bag until extraction. For each wether an aliquot of the faecal sample from each collection period was extracted and purified as described by Mayes et al. (1986), with the modifications that duplicate 0.5g samples were directly heated in ethanolic potassium hydroxide and 7ml of heptane were used as the solvent. To determine the amounts of n-alkane in the dose, duplicate 1g samples of each of the two diets and four particle pools were extracted the same way as faeces except that about 50% more solvent and water were used than in faecal extraction.

The final eluates were injected onto a capillary column (Hewlett Packard (HP) .5 Crosslinked Phenyl Methyl Silicone, 50m X 0.32mm X 0.17μm) in a model HP6890 gas chromatograph fitted with a flame ionization detector and linked to a PC run by an HP Chemstation software. The oven temperature programme was initially 65^0C and then rose to 300^0C at 10^0C/min where it was held for 20 min. The temperatures of the injection and detector ports were 325^0C and 350^0C, respectively. The flow rate of the carrier gas (N_2) was 33.4ml/min. and that of the makeup gas (N_2) was 24ml/min.

Identification of the different n-alkanes was made based on the relative retention times of known standards. The ratio of the peak areas of the analysed n-alkanes to that of the internal standard (C_{34}) was used to calculate n-alkane amounts in the samples.

Statistical analysis

The two-compartment model with gamma-2 age dependency and gamma-1 age independence, abbreviated $G2 \rightarrow G1 \rightarrow 0$ or G2G1, described by Ellis et al. (1994) was fitted to the faecal excretion data using Genstat 5 (Lawes Agricultural Trust, 1998) statistical package. The generated fast rate parameter (λ), slow rate parameter (K_2) and time delay (Lag) for each of the four particle pools were used to calculate mean retention times of the four particle pools in the fast and slow turnover compartments and in the entire gastrointestinal tract. Mean retention time in the fast turnover compartment (MRT_1) was calculated as $2/\lambda$; mean retention time in the slow turnover compartment (MRT_2) was calculated as $1/K_2$; mean retention time in the total gastrointestinal tract (MRT_T) was calculated as $MRT_1 + MRT_2 + Lag$. MRT_1, MRT_2 and MRT_T of the four particle pools were compared by analysis of variance (ANOVA) procedure using the statistical package Genstat 5 (Lawes Agricultural Trust, 1998). The data were analysed as a split-plot design, with sheep and period intersection as the main plots and particle pools within the sheep and period intersection as the subplots.

Model choice

Several mathematical models are available to fit to faecal or duodenal marker excretion curves and yield estimates of digesta retention times in the whole or in various parts of the ruminant gastrointestinal tract. Some are premised on assumption that the ruminant gastrointestinal tract is essentially a one way flow, two compartment system which can be mathematically described by a biexponential expression with time delay (Blaxter et al., 1956; Grovum and Williams, 1973). The two exponential terms are then used to describe processes in the mixing compartments and the time delay to describe movement in the tubular sections of the ruminant tract.

Dhanoa et al. (1985) proposed a model that considers digesta flow through the ruminant gut as a multicompartmental exponential process. This model proposes that digesta flows from the rumen, abomasum and caecum are likely to be exponential processes and events within the rumen are exponential-type processes. They further postulated that although digesta movement through the tubular sections of the gut is probably best described by longitudinal diffusion, several exponential components could approximate it. This model has the advantage of allowing for inclusion of several compartments, which, it could be argued, is more biologically appropriate to describe the multistage processes regulating passage in the ruminal tract. However, like the models proposed by Grovum and Williams (1973) and Blaxter et al (1956), it assumes instantaneous mixing in the mixing compartments and equal probability of escape for digesta particles of all ages. However, results of several investigations have suggested otherwise. For instance, Evans et al (1973) observed that, with passage of time, digesta in the reticulorumen undergo physical changes which enable them to pass to postruminal segments of the gut. This view was later supported by Sutherland (1988), who observed a ruminal floating raft, which, he suggested, functions as a discriminating mechanism with high selectivity for large particles. Furthermore, Reid (1986) listed as many as 13 events that he suggested are involved in the passage of solid digesta from the reticulorumen to the abomasum.

Models to fit to faecal or duodenal excretion data are usually chosen on the basis of how well they fit the data (Bernard et al., 1998; Dhanoa, 1985; Pond et al., 1988). Whilst this is a valid reason, it should be balanced with how closely the description of its mathematical terms relate to the known biological processes associated with digesta movement. Once these are met, relative ease to fit should be the next selection criterion because differences in the curve-fitting process can lead to conflicting conclusions being made about the same data (Bernard et al., 1998; Dhanoa, 1985). Bernard et al. (1998) in their model validation study demonstrated that different modelling methods fitted to the same data set could yield similar estimates of mean retention times of digesta in the various segments of the gut. Lindberg (1985) also found no difference in the mean retention times of digesta in sheep calculated using the methods of Blaxter et al., (1956) already referred to above and the algebraic method devised by Thielemans et al., (1978).

In this study the two-compartment model with gamma-2 age dependency and gamma-1 age independence proposed by Ellis et al., (1979) and Ellis et al., (1994) was chosen because it seems consistent with currently held views that with time digesta particles in the rumen undergo physical changes like size reduction and density increase before their probability of flow from the rumen increases (Poncet, 1991).

Assignment of physiological processes to model components

The assignment of physiological processes or anatomical parts to components of the model was based on the hypothesis this study set out to investigate (Figure 1) and on suggestions from the results of other studies that made use of the model (Pond et al., 1988; Bernard et al., 1998). Mean retention time in the fast compartment (MRT_1) was assumed to be largely accounted for by processes like comminution, hydration and microbial colonisation, which feed particles undergo when they first enter the rumen. The rest of MRT_1 would be accounted for by post-duodenal mixing segments of the gut (Pond et al., 1988). However, it should be noted that there are differences among investigators in the way they envisage this parameter (MRT_1) to represent digestive processes (Mambrini and Peyraud, 1997). For instance, in one study the model estimated residence time in post-duodenal mixing segments has been found to be of similar magnitude to the actual residence time large particles spend in a rumination pool of the rumen (Ellis et al., 1994). That suggests apportioning MRT_1 equally between the processes like size reduction and hydration, to which digesta are subjected when they first enter the rumen and the digestive processes in post-duodenal mixing segments.

Mean retention time in the slow turnover compartment (MRT_2) would be descriptive of the residence time of small and dense (owing to rumination and fermentation) in "competition" to leave the reticulorumen (Ellis et al., 1994). The slow turnover compartment is envisaged as the rumen, but residence time in it may include time in the hindgut mixing compartments, depending on the type and amount of digesta mass already in the hindgut (Deswysen et al., 1989). For instance, Ellis et al. (1994) observed a residence time of between 1.5 to 5 h between the duodenum and rectum in Holstein steers fed on hay. According to Moore et al. (1992), estimates for this compartment should be similar among models which, like itself, assume age-independence for particulate rate of escape from this compartment. Mean retention time in the whole tract would be the sum of the retention times in the fast and slow turnover compartments and time delay (lag), the length of time it took for the marker to first appear in the faeces. Lag was envisaged to account for the movement of digesta in the non-mixing tubular sections of the digestive tract, as proposed by Pond et al. (1988).

Digesta entering the rumen is envisaged to be a continuum of particles from very fine to very large, which are conveniently represented in the flow diagram (Figure 1) by the small and large buoyant particle pools. With passage of time, the large buoyant particles may undergo hydration and fermentation, which will increase their buoyancy, but despite this their probability of escape will still not be very high until they have also undergone comminution to a size small enough to promote their passage. Alternatively, they will be comminuted (via ruminative chewing) whilst still buoyant, which will still not guarantee the highest probability of escape until they have reached, through hydration, a certain density threshold.

Therefore, according to Figure 1, the large, buoyant particle pool (LB) would have the longest mean retention time and the small, dense pool (SD) would have the shortest mean retention in the reticulorumen and in the whole tract. The intermediate pools, the large, low buoyancy (LD) and small, buoyant (SB), would have intermediate retention times in the reticulorumen and the whole tract. Differences in the derived mean retention times of the 4 particle fractions would be used to validate the hypothesis conceptualised in Figure 1.

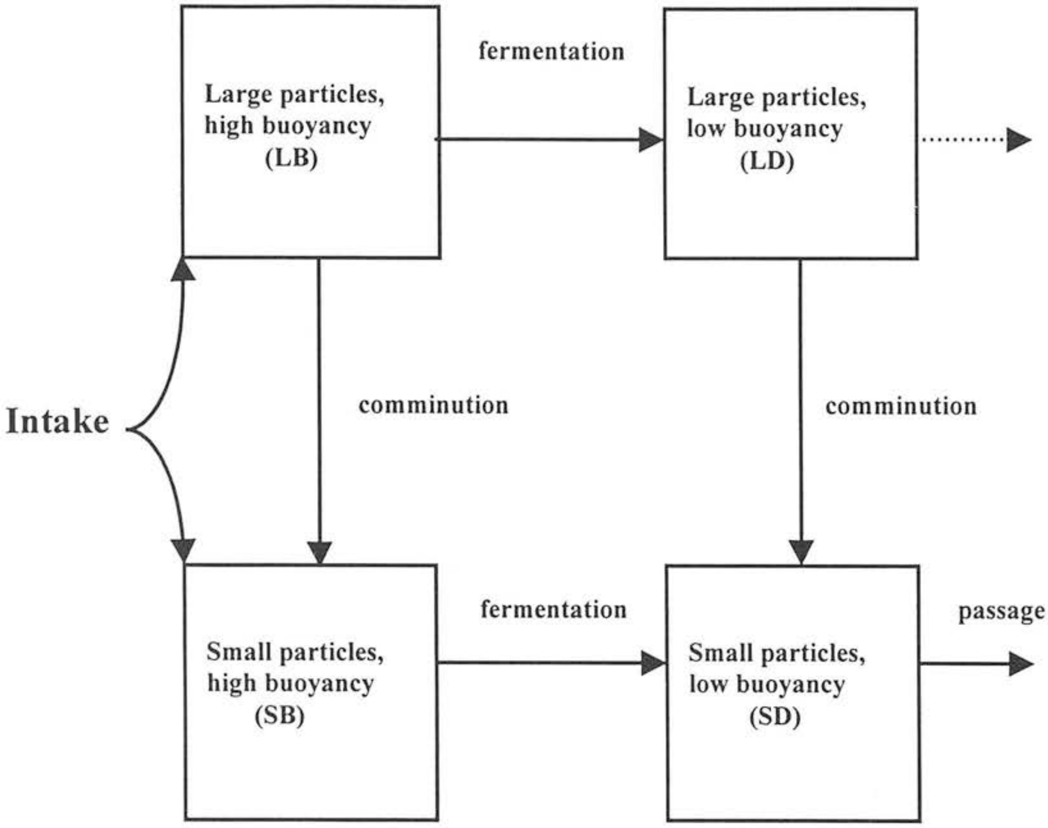

Figure 1. Schematic representation of digesta kinetics in the rumen

Results

Gas production experiment

The comparison of feed degradation parameters from *in vitro* gas production when various amounts of hexatriacontane (C_{36}) were used to coat 1 gram of ground hay is presented in Table 1. The asymptotic gas production from the fermentation of soluble carbohydrates (parameter A), the asymptotic gas production from the fermentation of NDF (parameter B) and the fractional rate of gas production per hour (parameter C) were not significantly different for the 4 amounts of C_{36} used. There was also no apparent difference between the lags (phases before the fermentation of NDF begins) of the hay fractions coated with 0, 12 or 24 mg of C_{36}. However, the lag phase of the hay fraction coated with 60mg of C_{36} was significantly longer ($p<0.005$) than those of the fractions coated with 0 and 12 mg, but not significantly longer than that of the fraction coated with 24 mg.

Table 1. Comparison of degradation parameters of ground grass hay coated with various amounts of hexatriacontane (C_{36}) per gram of hay (n=3).

	Parameters			
Alkane amount	A (cm^3)	B (cm^3)	C (/h)	Lag (h)
0 mg	13.41^{a}	41.63^{a}	0.0418^{a}	4.27^{a}
12 mg	12.58^{a}	41.53^{a}	0.0412^{a}	4.27^{a}
24 mg	13.50^{a}	40.67^{a}	0.0387^{a}	4.77^{ab}
60 mg	12.08^{a}	41.83^{a}	0.0387^{a}	5.23^{b}
SED	1.29	3.98	0.00274	0.30

For each parameter means with different superscripts within the same column differ significantly (P<0.05).

Rates of passage experiment

The mean retention times of the four alkane-marked particle pools, the large buoyant, small buoyant, large dense and small dense, in the fast turnover compartment, in the slow turnover compartment and in the whole gastrointestinal tract of the sheep are tabulated in Table 2. The large dense particles were held for the longest time (16.48h) in the fast turnover compartment and their retention time was significantly longer ($p<0.05$) than the retention times of the other particle pools in this compartment. The small dense particle pool was held for the shortest period (11.69h) in the fast turnover compartment and this retention time was significantly shorter ($p<0.05$) than that of the large buoyant pool, but not significantly shorter than that of the small buoyant pool. The mean retention times of the large and small pools were not significantly different from each other.

In the slow turnover compartment, the mean retention times of the large buoyant, small buoyant and small dense particles were not significantly different from one another (Table 2). However, the retention time of the large dense fraction, which was longest at 20.98h, was significantly longer ($p<0.05$) than those of the large buoyant and small buoyant fractions, but not significantly different from that of the small dense fraction.

In the whole tract, the mean retention time of the large dense pool was 58.08h and this was significantly longer ($p<0.05$) than the mean retention times of the other labelled particle pools. The small buoyant fraction had the shortest retention time at 46.50h, but this retention time was not significantly longer than that of the small dense fraction (49.58h). The retention time of the small dense was in turn not significantly different from that of the large buoyant fraction (57.75h), but the large buoyant fraction was held for significantly longer than the small buoyant fraction.

In order to facilitate the validation of the proposed model (Figure 1), the mean retention times of the particle pools shown in Table 2 were resolved so that they could bring to bear the effects of size and buoyancy on the length of retention of the particle fractions in the two compartments (fast turnover and slow turnover) and in the whole tract, and whether these were subject to dietary effects. However, the combined effects of both size and buoyancy were addressed in the discussion.

Table 2. Mean retention times (h) of the large buoyant, small buoyant, large dense and small dense particles in the slow turnover compartment (MRT_1), fast turnover compartment (MRT_2) and the whole gastrointestinal tract (MRT_T) (n=8)

	Particle pool				
Parameter	Large buoyant	Small buoyant	Large dense	Small dense	SED
MRT_1	14.20^{a}	12.70^{ab}	16.48^{c}	11.69^{b}	0.983
MRT_2	18.25^{a}	17.63^{a}	20.98^{b}	19.07^{ab}	1.038
MRT_T	51.75^{b}	46.50^{a}	58.08^{c}	49.58^{ab}	1.215

Mean retention times with different superscript letters within a row are significantly different ($p<0.05$)

The effect of particle size on mean retention time

In Table 3 the means of the mean retention times in the fast turnover compartment (MRT_1), slow turnover compartment (MRT_2) and total tract (MRT_T) for the large and small fibre particle pools are given. The small particle pool had a significantly

(p<0.001) shorter retention time in the fast turnover compartment than the large particle pool. In addition, there was a significant (p<0.005) diet and particle size interaction for MRT_1, which suggested that the difference in MRT_1 between the two particle pools was more pronounced when the sheep were fed on hay. The large and small particles were held for 12.3 and 11.59 h respectively when the sheep were fed on grassnuts compared to 18.38 and 12.80 h when they were fed on hay (Table 4).

There was no significant difference in the time the large and small particle pools were held in the slow turnover compartment (Table 3). However, there was a significant (p<0.001) diet and particle size interaction in MRT_2 such that the large particles were held 5 h longer than the small particles when the sheep were fed on grassnuts (Table 4). In contrast, the small particles were held 2.5 h longer when the diet was hay.

There was a significant (p<0.001) difference in retention time in the whole gastrointestinal tract between the large and small particle pools (Table 3). The large particles were retained almost 6 h longer than the small particles in the gastrointestinal tract. There was however no significant influence of diet on the retention time of the fibre particles in the whole tract

Table 3. Mean retention time (h) in the fast turnover compartment (MRT_1), slow turnover compartment (MRT_2) and total tract (MRT_T) for large and small particles (n=16)

	Mean retention times (h)		
Particle size	MRT_1	MRT_2	MRT_T
Large	15.34	19.62	54.91
Small	12.19	18.35	48.04
SED	0.695	0.734	0.859

Table 4. The influence of diet on MRT_1 and MRT_2 (h) of large and small particles (n=8)

Particle size	MRT_1 (h)		SED	MRT_2 (h)		SED
	Hay	Grass		Hay	Grass	
Large	18.38	12.30	[2]0.983	21.36	17.88	[2]1.038
Small	12.80	11.59		23.99	12.71	
SED	[1]1.297			[1]4.519		

[1]For comparing between diets within each MRT

[2]For comparing within diet within each MRT

The effect of particle buoyancy on mean retention time

Mean retention times in the fast turnover compartment (MRT_1), slow turnover compartment (MRT_2) and total tract (MRT_T) for buoyant and non-buoyant particles are presented in Table 5. There was no significant difference in the length of time the buoyant and non-buoyant particle pools were held in the fast turnover compartment. There was also no apparent dietary influence on the MRT_1 of the particles. There was however a significant ($p<0.05$) size and buoyancy interaction (Table 6). The large particles of the dense fraction had a 5h longer MRT_1 than the smaller ones of the same fraction. In a similar manner, the large particles of the buoyant pool were retained 1.5 h longer than the small particles.

The non-buoyant particles were retained in the slow turnover compartment 2 h longer ($p<0.05$) than their buoyant counterparts (Table 5). There was however no significant interaction between buoyancy and diet in MRT_2

There was a significant ($p<0.001$) particle buoyancy effect in the whole tract such that the non-buoyant pool remained in the tract 4.71 h longer than the buoyant pool (Table 5). There was no significant diet and buoyancy interaction in MRT_T

Table 5. Mean retention time (h) in the fast turnover compartment (MRT_1), slow turnover compartment (MRT_2) and total tract (MRT_T) for buoyant and non-buoyant particles (*n*=16)

	Parameter		
Particle buoyancy	MRT_1	MRT_2	MRT_T
Buoyant	13.45	17.94	49.12
Non-buoyant	14.08	20.02	53.83
SED	0.695	0.734	0.859

Table 6. Mean retention time (h) of buoyant and non-buoyant large and small fibre particles (*n*=8) in the fast turnover compartment

	Mean retention time (h)		SED
Particle size	Buoyant	Non-buoyant	
Large	14.20	16.48	
			0.983
Small	12.70	11.69	

Discussion

Gas production

The *in vitro* gas production technique has been used to determine the kinetics of ruminant feedstuff degradability from gas produced by samples of feedstuffs that are fermented in buffered rumen fluid kept at 39 ^{0}C (Menke et al., 1979; Pell and Schofield, 1993). Based on the pattern of its fermentative gas production, degradability kinetics and apparent in vivo digestibility of a feed can be predicted (Menke et al., 1979; Romney et al., 1998). Blümmel and Ørskov (1993) found the total gas produced by their gas production system to be correlated with DM intake, digestible DM intake and animal growth rate. Because of these attributes and that the technique is inexpensive and not labour-intensive, it was adopted to investigate the effects of coating feed particles with increasing amounts of alkanes on *in vitro* degradation characteristics.

The results of this study suggested that up to 24 mg of alkane could be used to coat 1 gram of feed without significantly affecting the measured degradation parameters of the feed. Coating 1 gram of feed with 60 mg did not seem to affect the amount of gas produced in the first 4 h, the total amount of gas produced and the rate of gas produced. Although the lag phase of the grass hay coated with 60 mg of alkane was slightly longer than those of the feeds coated with 12 mg of alkane or uncoated, it was not significantly longer than the lag phase of grass hay coated with 24 mg. It was therefore concluded that using up to 50 mg of alkane to coat 1 gram of fibre particles would not appreciably alter their kinetics in the ruminant gastrointestinal tract, particularly for purposes of comparing passage rates of different particles, provided they are all marked with the same concentration of alkane.

Rates of passage

The effect of particle size on mean retention time

The longer mean retention of the large particles than of the small particles in the fast turnover compartment (Table 4) found in this experiment is in agreement with the general observations by similar studies that mean retention time increases with an increase in particle length (Poppi et al., 1980; Murphy and Kennedy, 1993; Welch, 1982; Ellis et al., 1994). The longer retention time for the large particles could be attributed to the time it took to reduce them to a size small enough to allow easy passage through the reticulo-omasal orifice (Mambrini and Peyraud, 1997). Evidence of particle reduction during passage was also presented in a study to compare digesta particle size distribution in the reticulorumen, omasum and abomasum of sheep fed on two alfalfa hay varieties and four grass hay types (Troelson and Campbell, 1968). They found a higher proportion of coarse particles in the reticulorumen than in the other compartments. Bernard et al. (1998) evaluated 3 curve-fitted models, including the one used in this study, against an algebraic method and found longer mean retention times for thulium (Tm)-labelled chopped than ytterbium (Yb)-labelled ground (8 mm screen) orchardgrass hay in the stomach and whole tract of sheep. They estimated comminution time of large particles in the rumen to be the difference between the retention time of the small particles and the retention time of the chopped hay and found this to be 15.9 h when estimated from faecal data. If the same method is employed when the sheep were fed on hay in the present study, comminution time for the large particles is 5.6 h, which is much shorter than the 15.9 h reported by Bernard et al. (1998). Chopping, as was done Bernard et al. (1998), would be expected to produce larger particles which would take longer to comminute than milling through a 6 mm screen, hence the discrepancy in the comminution time estimates of the two studies.

Particle size reduction due to ruminative chewing is influenced by factors like plant characteristics, time after feeding and level of intake (Kennedy and Doyle, 1993; Murphy and Kennedy, 1993). One or more of these factors could have caused the difference in the MRT_l values of the two studies. Furthermore, that the two particle fractions were marked with 2 different rare earth markers, could have conceivably altered the behaviour of the particles they were intended to mark (van Soest et al., 1988) and influenced mean retention times differently. In contrast, Mambrini and Peyraud (1997) who also used rare earth markers, estimated that the difference in mean retention times of long and ground hay in the whole tract of dairy cows to be 7 h and attributed this difference to the time needed to reduce the long forage to the length of the ground particles.

In this study the large and small particles were held in the fast turnover compartment for 12.3 and 11.59 h, respectively, when the sheep were fed on grassnuts compared to 18.38 and 12.80 h (Table 4) when they were fed on hay. A longer mean retention time for fescue hay compared with a commercial pelleted diet were also found by Moore et al. (1992) who investigated the influence of different markers and models on digesta passage rates when sheep were fed on either of the two diets. The longer retention of the large particles when the sheep were fed on bailed hay could be due to their entrapment in the floating raft proposed by Sutherland (1988), which forms in the rumen after ruminants have consumed fibrous feed like hay. In the case of the present study, pelleting of the grass (grassnuts) may have eliminated or reduced the formation of the floating raft and thus allowing a higher rate of escape for large particles that would otherwise be entangled in the raft (Van Soest et al., 1988; Kennedy and Doyle, 1993).

Alternatively, pelleting (grassnuts) may have enhanced the intake of grass by the sheep and as a consequence the rates of passage of all feed residues associated with it (including the large marked particles) increased (Okine and Mathison, 1991). According to Luginbuhl et al. (1990), citing earlier work, reducing dry matter intake of Coastal bermudagrass by steers to 50% of ad libitum level increased gastrointestinal tract retention time of masticated boli, leaves and stems by 24, 22 and 25 h, respectively, when compared with near ad libitum feeding. Further, Blaxter et al. (1956) compared the digestibilities and retention times of long dried grass, the same grass medium ground and cubed and finely ground and cubed in the digestive tracts of sheep fed at different levels of intake. Results indicated that the cubes of dried grass passed along the tract more quickly than the long material and that increasing intake level also resulted in an increase in the passage rates of the grasses. Therefore the small difference between the mean retention times of the large and the small particles when the sheep were fed on grassnuts was not inconsistent with lower retention of large particles, having escaped rumination and raft entrapment, that is sometimes shown with higher intake (Van Soest et al., 1988).

However, grinding of feedstuffs may increase their retention time in the digestive tract. For instance, unlike in the present study, Faichney (1983) found that grinding and pelleting lucerne hay increased the mean retention times of solutes and of particle-associated marker in the rumen of sheep. Similar findings were also reported by Weston and Hogan (1967) who observed a reduction in the flow rate of particulate matter and of water from the rumen of limited-fed sheep when chopped lucerne was replaced by an equal amount of ground lucerne. This reduction in the flow rate of particulate matter from the rumen was attributed to a possible increase in organic matter content of digesta in the reticulum, which slows flow to the omasum when ground feeds are given.

Further support for the size reduction theory in the fast turnover compartment is lent by the lack of significant difference in MRT_2 of the large and small particles (Table 3), which, in line with the proposed model, would suggest that the particles in the slow turnover compartment were mostly equal in size (small). However, the diet and particle size interaction in MRT_2, such that the large particles were held 5 h longer than the small particles when the sheep were fed on grassnuts while the large particles were held only 2 h longer when the sheep were fed on hay is difficult to explain. Theoretically, in this compartment, particles have been reduced to a size small enough to allow passage and all have an equal probability of passage through the reticulo-omasal orifice (Ellis et al., 1994). However, even with those particles considered to be eligible in terms of size for passage from the reticulorumen, passage rates can differ substantially (Murphy and Kennedy, 1993). Evidence for possible existence of another sorting mechanism beyond the reticulorumen, by which large particles were selectively retained and returned to the reticulum, was observed by McBride et al. (1984) in a cow fed alfalfa hay and by Waghorn et al. (1986) in sheep fed chaffed lucerne. Therefore some large particles which may have escaped rumen entrapment as a result of feeding grassnuts could have been selectively returned to the reticulum or retained in the omasum and hence the longer MRT_2. The small particles, on the other hand, passed through relatively unhindered.

The large particles were retained 7 h longer than the small particles in the whole gastrointestinal tract (Table 3). This result was not unexpected and is consistent with differential retention for large and small particles reported by Quiroz et al (1988) with goats fed Coastal bermudagrass or orchardgrass hay and by Linberg (1984) with sheep given hay and a concentrate diet at maintenance feeding level. Differential mean retention times of the large and small particle fractions in the whole tract should be mainly accounted for differential retention times of these two fractions in the fast turnover compartment, which, according to the model proposed here, takes place mainly in the reticulorumen and the caecum-colon. The relative contribution of each of these mixing segments to the MRT_1 may be almost equal (Pond et al., 1988; Bernard, 1998) or the reticulorumen may account for most of MRT_1 (Ellis et al., 1994). However, because there was no duodenal sampling done in this study, resolving the estimated MRT_1 into retention times in each of the mixing segments would be difficult and no attempt was made to partition MRT_1 between these anatomical parts. Therefore the difference of 3 h between the retention of the large and small particles accounts reflects collective particle comminution in the mixing compartments. If that were the case, and the results of this study suggested that there was no significant difference in the MRT_2 of the large and small particle fractions, then the 7 h difference in the MRT_T of the large and small fractions suggests a further 4h delay of the large particles in the tubular segments of the tract. Such a differential delay was unexpected as the influence of size on particulate flow is less important in the postruminal segments of the gut (Murphy and Kennedy, 1993; Kaske and Engelhardt, 1990), unless there were substantial differential mean retention times for different digesta, as was proposed by Barry et al. (1985).

The difference could be due to the involvement of a density- and size-related sorting mechanisms, particularly in the omasum and abomasum, gut segments not thought to contribute much to gut retention time. It has been shown that small particles undergo a higher rate of density change than large particles (Hooper and Welch, 1985) and this differential density change could have delayed the first appearance of the large particles in the faeces. However, Campling and Freer (1962) observed a slower passage rate postruminally as density increased. It may also be due to deviations of biological systems from the ideal mathematical assumptions of continuous flow and instant mixing in the system. For instance, defecation is not always regular and this can influence retention in the whole tract or first appearance of marker in the faeces. Also, passage of small particles may be accelerated during eating (Kennedy and Doyle, 1993; Ellis et al., 1999) and under ad libitum feeding conditions, which can be a source of variability

The effect of buoyancy on mean retention time

The results of this study did not suggest any discernible overall effect of buoyancy on the mean retention time of the particles in the fast turnover compartment (Table 5). Such a result contradicts the postulation made by Kaske and Engelhardt (1990) that rates of passage in sheep are influenced more by particle density than by particle size. By contrast the result supports the findings of Kennedy (1995) who observed no obvious effect of buoyancy on the rate of passage of particles out of the rumen of swamp buffaloes and cattle. For this study, however, that result was unexpected as the particle fractions labelled non-buoyant were expected to be dense as a result of soaking for 7 days in rumen fluid. The density acquired by the particles was not quantified, but published data suggest that it would have been about 1.3g/ml (Hooper and Welch, 1985) or 1.5 g/ml (Evans, 1973). This density would be at or slightly higher than the upper limit of the density range that has been observed to promote the most rapid particulate passage by many studies (Kaske and Engelhardt, 1990; desBordes and Welch 1984; Murphy et al., 1989). Thus incubating the feed particles for as long as they were in the present study may have made them too dense for rapid flow from the rumen once they had hydrated. As a consequence the time it took them to finally exit the reticulorumen was similar to the time it took the unincubated fraction to undergo the time-dependent ruminal processes which increase their density and thus their probability to escape out of the reticulorumen.

There are other possible reasons to explain the apparently similar rates of passage for the buoyant and non-buoyant fractions in the fast turnover compartment. MRT_1 represent compartmental mixing flow in the rumen as well as in the postruminal segment as was suggested by the results of Pond et al. (1988) who reported about 40 % longer MRT_1 estimates from duodenal than from faecal excretion curves of cows fed mainly chopped or pelleted straw. Without marker concentration data from other parts of the digestive tract it would be difficult to accurately resolve this mean retention time into the times spent in each compartment. Furthermore, MRT_1 is closely related to time delay such that an overestimation of either parameter results in an underestimation of the other (Ellis et al., 1994), which means that difference in

MRT_l between large and small particles could have been masked by inaccuracies in estimating time delay. Similar difficulties have been reported by other investigators (Lallès et al. 1991); Faichney and Griffiths (1978) resolved to combine the estimates of the two parameters. It was difficult to fit models to a few of the excretion curves and the curves that fitted gave similar rates of exit for both slow and fast turnover compartments, a scenario which, according to Pond et al. (1988), violates the assumptions of the $G1 \rightarrow G2 \rightarrow 0$ model and casts a doubt on the generated estimates. Maybe for those curves another model should have been sought as was suggested by Quiroz et al. (1988), but that would have made comparison between estimates even more difficult.

The effect of buoyancy on particle mean retention time was more noticeable in the slow turnover compartment and in the whole tract (Table 5). The effect of buoyancy also came through in the size and buoyancy interaction in the fast turnover compartment (Table 6), such that for the non-buoyant fraction the large particles had a much longer retention time than the small ones, whereas for the buoyant fraction the large and small particles had similar retention times. As previously stated, the purpose of incubating the non-buoyant pools in rumen liquor was to increase their specific gravity so as to facilitate their movement along the digestive tract. It would appear therefore that the dense particles, on entering the reticulorumen quickly sedimented to the bottom. The large particles, hampered by their large size, could not easily go through the reticulo-omasal orifice. Their high density which meant that they sank to the bottom of the reticulorumen also made them less likely to be aspirated to the mouth for ruminative chewing and thus size reduction which would promote their chances of passage through the orifice. The predominant size-decreasing mechanism to which they (large dense) were subject was detrition, which is a slower process than ruminative chewing. Therefore the onward passage of the incubated large particles was hindered by both size and density, hence the 5h difference in MRT_l between the large and small particles of the non-buoyant fraction (Table 6). In contrast, the LB fraction, because of its low density, did not separate from the main floating digesta mass and was aspirated into the mouth for ruminative chewing and attained the optimum density, which made it to have a shorter MRT_l than the LD fraction.

If MRT_l is assumed to largely represent processes like size reduction, hydration and density increase, which particles undergo on entering the reticulorumen, then the small non-buoyant particle (SD) pool would be expected to have a shorter MRT_l than the larger particle (LD and LB). However, the results of this study suggest that MRT_l for the large and small buoyant pools approximated that of the small non-buoyant pool, which implies that the onward movement of the small non-buoyant particles was also somewhat hindered by unanticipated processes. A possible cause of that delay is that on hydration the SD increased density beyond the optimum range for easy passage through the reticulo-omasal orifice. Beside the short time required for their hydration, their MRT_l would then be accounted for by the amount of time needed to take them from where they were deposited to where they sedimented in the rumen and finally to a favourable position in then reticulum for onward travel to the omasum.

The result of a shorter MRT_l for the large buoyant than for the large non-buoyant pool (Table 6) was not expected because incubation was meant to increase density and consequently rate of passage, but similar results have been reported with mordanted fibre in cattle elsewhere. For instance, Ehle et al. (1984) and Ehle (1984) reported an increase followed by a decrease in ruminal passage rates of alfalfa fibre particle densities in Holstein cows, when increased by mordanting with increasing concentrations of chromium. Campling and Freer (1962) concluded that although plastic particles of specific gravity of 1.40 readily separated from the main mass of digesta were not as readily transported in the liquid digesta leaving the reticulo-rumen.

Another possible explanation for the buoyancy and size interaction in the fast turnover compartment could be that the large and small particle pools of the buoyant fraction may not have been as well defined as those of the non-buoyant fraction. The non-buoyant fraction, after harvesting from rumen liquor, was strained in two layers of muslin and rinsed repeatedly in cold water, which would have washed out most fine particles in both fractions and left no continuum between the two sizes. The large buoyant fraction could have had finer particles than were intended, which could have migrated ahead of the large buoyant fraction and masked size effects in this fraction (Faichney et al. 1989). Also, the rate of change of specific gravity in vitro was shown to be related to particle size, with small particles changing more quickly than large particles (Hooper and Welch, 1985). Therefore in well-defined large and small fractions, as was the case with the fermented particles, the difference in the flow rate of large and small particles possibly reflects more than in the less well-defined non-fermented fraction the differential sedimentation rates between large and small particles.

Suitability of alkanes as markers for rates of passage of solid digesta

The most commonly used methods for measuring passage rates involves administration of indirect markers (Faichney, 1993). Criteria for the ideal marker have been suggested and include inertness, noninterference with the digestive or physiological processes, physical similarity to or intimate association with the fraction to be marked, recoverability and easy quantification (Kotb and Luckey, 1972; Faichney, 1993). However, none of the markers in use today meets all the desirable characteristics (Dove and Mayes, 1991). Although the results of the gas production and passage rate experiments reported here do not prove that alkanes meet all the criteria for the ideal marker, they go along way towards proving the superiority of alkanes as markers for estimating solid digesta passage rates.

The results of the *in vitro* gas production study suggested that attaching up to 24 mg of alkane per gram of forage compared favourably with not treating because the measured degradation parameters of the alkane-coated feed (up to 24 mg) were not significantly different from those of the untreated forage (Table 1). Coating 1 gram of feed with 60 mg of C_{36} did not seem to affect the amount of gas produced in the first 4 h, the total amount of gas produced and the rate of gas produced. However, the lag phase of the forage coated with 60 mg was longer than those of the fractions coated with 0 and 12 mg, but not significantly longer than that of the fraction coated with 60mg. This would seem to suggest that up to 24 mg of alkanes can be safely used to mark 1 gram of forage without compromising the degradation characteristics of the feed that are measurable by the *in vitro* gas production technique, which would lend credence to the suitability of alkanes as markers for investigating solid digesta passage rates.

Another characteristic that influences the digestion kinetics of digesta in the digestive tract is buoyancy or density. The amount of alkanes (up to50 mg per gram of forage) used here are unlikely to alter appreciably the density and thus the passage rate of the forage to which they are attached.

The results of these experiments could not conclusively prove that alkanes stick and remain attached to the particles to which they were attached. However, after the forage was coated by soaking in alkane solution, the alkane-coated forage was oven-baked at 100^0C for 1 hour to melt the alkanes into the forage. This oven treatment should have rendered the alkanes intimately attached to the forage particles.

There was also no obvious evidence of alkanes dissociating from the particle fraction to which they were originally attached and migrating to other fractions. Four particle pools expected to have different kinetics (due to different physical characteristics) in the gastrointestinal tract were coated with four different alkanes and dosed to the sheep, and all the four alkanes were detectable in the faeces and the results suggested differences in the kinetics of the four particles pools in the digestive tract. Although not conclusive, these results would seem to suggest that the attached alkanes did not migrate to particles to which they were initially not attached. This result would also suggest that the alkanes remained associated with the particles to which they were originally attached even after comminution due to ruminative chewing and detrition.

Several questions regarding the use of alkanes as markers for estimating solid digesta rates of passage have not been answered by the results of the study reported here. For instance, how alkanes of different chain length would behave in the gastrointestinal tract and how a difference in behaviour would influence passage rates. One way of investigating that would be comparing the passage rates of each of the four particle pools after coating with alkanes of different chain length.

Conclusion

Although the results of this experiment do not fully validate the proposed model of solid digesta kinetics in the rumen (Figure 1), they reinforce the model expectation that small particles would pass faster than large particles along the digestive tract. The role of diet in influencing digesta movement was brought out, such that on the ground diet (grassnuts) differences between the large and the small particles were not as pronounced as they were when the sheep were fed on hay. There are two probable reasons for this. On the one hand, is more prolonged entrapment of the larger particles in the floating digesta mat which forms after ruminants have eaten fibrous feeds like hay. On the other, is higher dry matter intake resulting from milling and pelleting (grassnuts), which usually promotes rapid rates of passage of digesta and no fibrous mat formation. This scenario would promote a higher rate of passage of particles which would otherwise be held in the raft when there is one. Therefore the findings of this study are consistent with the expectation that digesta particles have to be broken down to some threshold size of fineness before passing from the rumen, but diet type may modify the rate of passage (Van Soest et al., 1988; Wilson and Kennedy, 1996).

In agreement with the proposed model the results of this study suggested the involvement of specific gravity in particle passage, but not in the manner depicted in the model. Incubating fibre particles in rumen liquor appears to have made them too dense to readily migrate out of the reticulorumen ahead of the non-fermented particles; instead their passage was retarded so much so that the non-fermented particles passed more rapidly out of the rumen and in the whole tract. It would be interesting to measure the specific gravities of particles that have been incubated in rumen liquor for varying lengths of time.

Another possibility is that fermentation in rumen liquor should have depleted the digestible material in the non-buoyant fractions and therefore the buoyant and non-buoyant fractions should have been chemically dissimilar. The capacity of forages to absorb water and increase in density is related to their chemical characteristics (Martz and Belyea, 1986). Forages with a high content of structural material like cellulose and lignin are expected to hydrate slower than high quality forages (Martz and Belyea, 1986). It is therefore plausible that the fermented material used in this study resisted hydration and thus density increase because lignin, which has a low affinity for water, was the predominant material left in them after fermentation in rumen liquor. Consequently, the non-fermented fractions may have rehydrated, increased in density and passed faster than the fermented particles.

CHAPTER 6

Introduction

Voluntary intake of roughages by ruminants is modulated by many interrelated animal and feed factors, such as the physiological state of the animal and the chemical and physical nature of the roughage source. Ruminants are able to vary rumen capacity and particle outflow rates and thus intake according to physiological need. For instance, lactating cows increase rumen fill and passage out of the rumen of feed residues in order to meet the higher nutrient and intake demands of lactation (Ellis et al., 1999).

Animals of different ages would be expected to have different physiological needs and thus food intake requirements. Intake-related attributes like bite mass have been found to be allometrically related to animal body mass (Illius and Gordon, 1999), but there are limited data which relate intake attributes to changes in animal body mass within species. There are also mechanistic models which integrate animal size and feed characteristics to predict food intake by ruminants, e.g. Illius and Gordon (1991), but these have not been validated using a range of animal sizes within a species. It would also be beneficial to modelling to delineate how intake-related attributes like rumen fill and passage rates of particles of various characteristics change in relation to changes in body mass as animals grow. The objectives of this experiment were thus to compare 1) the passage rates of fibre particles of two sizes and two densities in the gastrointestinal tracts of four sizes of a tropical cattle breed, Criollo and the rates of passage of the same fibre particles in the mature tropical breed against a similarly sized Criollo X Holstein cross and 2) the voluntary dry matter intake and gutfill in the afore-mentioned cattle sizes and genotypes.

Materials and methods

Animals and feed

Passage rates and intake

Sixteen Criollo bulls were arranged by weight into 4 groups of 4 animals. The first group (Group 1) had an average weight of 87.5kg, the second (Group 2) had an average weight of 146.25kg, the third (Group 3) had an average weight of 310kg and the fourth (Group 4) had an average weight of 446kg. An additional group of 4 Holstein X Criollo bulls (Group 5) of average weight 445kg was used for breed comparison with Group 4. Groups 1 and 2 were mainly grazing *Brachiaria decumbens* in a 2 ha plot away from their mothers, but were allowed to suckle for about 1h after milking in the morning and in the afternoon. All the other groups were also grazing *Brachiaria decumbens* in a separate 7.5 ha plot.

Dose preparation

Passage rates

Two portions of *Brachiaria decumbens* from the same plot in which Groups 3, 4 and 5 were grazing during the trial were previously harvested and hayed before grinding through a hammer mill, one to pass through a 1 mm screen and the other to pass through a 6 mm screen. The fraction that was ground through a 1 mm screen, which was labelled the small, buoyant pool (SB), was marked with n-octacosane (C_{28}) at a concentration of 50 mg of C_{28} per gram of hay. The results of a gas production study (Chapter 5) suggested that perhaps up to 50 mg of alkane could be used to mark 1 g of feed without adversely affecting the *in vitro* degradation characteristics of the feed. The marking was achieved by dissolving the required amount of n-alkane in n-heptane heated to 80^0C and soaking the hay in the n-alkane solution while continually stirring for about 15 minutes. The hay was then dried in a fume cupboard for at least 24h to evaporate the n-heptane and oven-baked at 100^0C for 1h to melt the n-alkanes into the hay.

About 1/4 of the hay ground through a 6mm screen, which was labelled the large, buoyant pool (LB), was marked with n-hexacosane (C_{26}) using the same procedure used to mark SB. The remainder was incubated for 120h in a mixture of 1 part strained rumen liquor and 3 parts buffer solution (Menke et al., 1979) contained in flasks stoppered with fermentation locks and kept in a water bath at 39^0C. The flasks were shaken 4-6 times a day. As a general guide, about 800ml of the rumen liquor and buffer solution mixture were enough to incubate 15 grams of grass. At the end of the incubation period the rumen liquor was strained out through 2 layers of muslin and discarded while the hay was collected and oven-dried at 60^0C for 48 hours before it was separated into two approximately equal fractions. One, which was labelled the small, dense pool (SD) was ground to pass through a 1mm screen in a hammer mill and then marked with n-dotriacontane (C_{32}); the other, which was labelled the large, dense pool (LD) was marked with n-triacontane (C_{30}) without grinding. Both fractions were marked with n-alkanes following the same procedure used for the unfermented hay and were also marked at the concentration of 50 mg of alkane per gram of hay.

Intake

The bulls were dosed with n-hexatriacontane (C_{36}) impregnated into a cotton filter prepared as described below.

Cotton filters were loosened by gently rolling between finger and thumb and placed in aluminium foil trays which were later placed in oven at 100^0C for 30 minutes. A stock solution of n-hexatriacontane (C_{36}) in n-heptane at a concentration of 1g per 10ml was prepared in a volumetric flask. The flask was placed in a water bath at 60^0Cand shaken until the all the alkane dissolved. About 200ml at a time were transferred into a smaller flask suspended in the water bath by the neck, using a clamp and a retort stand. An aliquot of the alkane solution enough to carry 200mg (2ml) of alkane was carefully dispensed with a positive displacement pipette onto each filter, ensuring that the solution was absorbed into the filter. The filters were placed in a fume cupboard overnight to evaporate the solvent and the next day they were placed in an oven at 100^0C for 20 minutes to melt the alkane into the filters. The same method was used to impregnate smaller filters for Group 1 with 100mg (1ml) of hexatriacontane per filter.

Dosing and faecal sample collection

Passage rates

Ten grams (marked with 500mg of n-alkane) of each of the four particle pools were weighed into tissue paper and dosed once orally to Groups 3, 4, and 5; group 1 and 2 were dosed with 5 grams (marked with 250mg of n-alkane). Faecal grab samples were collected first before dosing and thereafter at 6 hourly intervals for 106 h and at 12-hourly intervals until 138 h.

Intake

Each animal in Groups 2, 3, 4 and 5 was dosed orally once a day (at 06:00) over a 12 day period with 400mg (2 X 200mg) of n-hexatriacontane (C_{36}) impregnated into a cotton filter. Animals in Group 1 were dosed in the same manner but with 200mg (2 X 100mg) of n-hexatriacontane. From day eight faecal grab samples were collected at 6 hourly intervals for five days from each bull (these were the same samples collected for passage rates measurement in the same period),

Extraction of faecal and dose samples

Passage rates

The collected faecal samples were oven dried at 60^0 C for 48 hours and then ground in a coffee grinder before extraction and purification. For each bull an aliquot of the ground faecal sample from each collection period was extracted and purified as described by Mayes et al. (1986), with the modifications that duplicate 0.5g samples were directly heated in ethanolic potassium hydroxide and 7ml of heptane were used as the solvent. To determine the amounts of n-alkane in the dose, duplicate 1g subsamples of hand-plucked samples from each of the two grazed plots and samples from the four particle pools were extracted the same way as faeces except that about 50% more solvent and water than in faecal extraction were used. The final eluates were analysed for alkane amounts by gas chromatography.

Intake

Faecal and hand plucked samples taken from each of the two grazed plots were extracted the same way as in the passage rates method. Several filters from each of the two batches (100 mg and 200 mg) that were used for dosing the bulls were extracted to determine the average amount that was adsorbed onto each filter. The method involved slicing three pellets and boiling them, together with 100mg of internal standard (C_{34}), under reflux in 100ml of heptane for 1h (Mayes unpublished protocol). After partial cooling to about 60^0C an aliquot was taken and analysed by gas chromatography.

Chromatographic analyses

Passage rates and intake

The samples to be analysed were injected onto a capillary column (Hewlett Packard (HP) .5 Crosslinked Phenyl Methyl Silicone, 50m X 0.32mm X 0.17µm) in a model HP6890 gas chromatograph fitted with a flame ionization detector and linked to a PC run by an HP Chemstation software. The oven temperature programme was initially 65^0C and then rose to 300^0C at 10^0C/min where it was held for 20 min. The temperatures of the injection and detector ports were 325^0C and 350^0C, respectively. The flow rate of the carrier gas (N_2) was 33.4ml/min. and that of the makeup gas (N_2) was 24ml/min Identification of the different n-alkanes used as markers and those needed for intake estimation was made based on the relative retention times of known standards. The ratio of the peak areas of the analysed n-alkanes to that of the internal standard (C_{34}) was used to calculate n-alkane amounts in the samples.

Model choice and description

Passage rates

The two-compartment model with gamma-2 age dependency and gamma-1 age independence, abbreviated G2→G1→0 or G2G1, described by Ellis et al. (1994) was fitted to the data using Genstat 5 (Lawes Agricultural Trust. 1998) statistical package. This model was chosen because it seems consistent with currently held views that with time digesta particles in the rumen undergo physical changes like size reduction and density increase before their probability of flow from the rumen increases (Poncet, 1991).

The assignment of physiological processes or anatomical parts to components of the model was as described in the materials and methods section of Chapter 4 of this manuscript. Briefly, mean retention time in the fast compartment (MRT_1) was assumed to be largely accounted for by processes like comminution, hydration and microbial colonisation, which feed particles undergo when they first enter the rumen, with the rest of MRT_1 can be accounted for by post-duodenal mixing segment of the gut. Mean retention time in the slow turnover compartment (MRT_2) would be descriptive of the residence time of small and dense (owing to rumination and fermentation) in "competition" to leave the reticulorumen (Ellis, 1994). Mean retention time in the whole tract would be the sum of the retention times in the fast and slow turnover compartments and time delay (lag), the length of time it took for the marker to first appear in the faeces. Lag was envisaged to account for the movement of digesta in the non-mixing tubular sections of the digestive tract, as proposed by Pond et al. (1988).

Digesta entering the rumen is envisaged to be a continuum of particles from very fine to very large, which are conveniently represented in the flow diagram by the small and large buoyant particle pools (Figure 1, Chapter 5). With passage of time, the large buoyant particles may undergo hydration and fermentation, which will increase their buoyancy, but despite this their probability of escape will still not be very high until they have also undergone comminution to a size small enough to promote their passage. Alternatively, they will be comminuted (via ruminative chewing) whilst still buoyant, which will still not guarantee the highest probability of escape until they have reached, through hydration, a certain density threshold.

Therefore, according to Figure 1 of Chapter 5, the large, buoyant particle pool (LB) would have the longest mean retention time and the small, buoyant pool (SD) would have the shortest mean retention in the reticulorumen and in the whole tract. The intermediate pools, the large, low buoyancy (LD) and small, buoyant (SB), would have intermediate retention times in the reticulorumen and the whole tract.

Differences in the derived mean retention times for the 4 particle fractions would be used to validate the hypothesis conceptualised in Figure 1 of Chapter 5.

Data analyses

Passage rates

The generated fast rate parameter (λ), slow rate parameter (K_2) and time delay (Lag) for each of the four particle pools were used to calculate mean retention times of the four particle pools in the fast and slow turnover compartments and in the entire gastrointestinal tract. Mean retention time in the fast turnover compartment (MRT_1) was calculated as $2/\lambda$; mean retention time in the slow turnover compartment (MRT_2) was calculated as $1/K_2$; mean retention time in the total gastrointestinal tract (MRT_T) was calculated as $MRT_1 + MRT_2 + Lag$. MRT_1, MRT_2 and MRT_T of the four particle pools were compared by analysis of variance (ANOVA) procedure using the statistical package Genstat 5 (Lawes Agricultural Trust. 1998). The data were analysed as a split-plot design, with cattle as the main plots and particle pools within the animals as the subplots.

Intake

Dry matter intake for each animal was estimated from the concentrations of n-alkanes (C_{36} and C_{35}) in the faeces and the consumed feed according to the method described by Mayes et al. (1986). The derived intake data were analysed as a completely random design and intake between groups were compared by ANOVA using Genstat 5 (Lawes Agricultural Trust. 1998).

Results

Influence of particle size

The mean retention times of large and small particles in the fast turnover compartment (MRT_1) for each of the five cattle groups are presented in Table 1. Across all the groups (sizes and breeds) large particles were held longer ($p<0.001$) than small particles in the fast turnover compartment. There was however no detected influence of either animal breed or animal size.

Table 1. Comparison of fast turnover compartment mean retention time (MRT_1, h) of large and small particles for each cattle group.

Particle size	[1]Animal Group					SED
	1^{I}	2^{II}	3^{III}	4^{IV}	5^{IV}	
Large	11.8	11.50	9.75	12.30	11.48	
						2.008
Small	8.05	6.02	5.00	7.11	7.86	

[1]Groups 1-4 are Criollo and Group 5 are Holstein x Criollo. To compare between breeds take average of Groups 1-4 and compare with group 5 using SED in table and 25 d.f.

[I-IV]Animal size; for comparing means within the same size, take average of groups 4 and 5 and use SED=1.395 with d.f.=45. Otherwise use SED in table and 25 d.f.

The mean retention time of the small particles in the slow turnover compartment were on average shorter ($p<0.001$) than those of the large particles. However, there was no significant difference in the retention times of the large and small particles in the slow turnover compartment of size II (Table 2). There were no significant interactions of animal size and particle size and of breed and particle size evident from the data.

Table 2. Comparison of slow turnover compartment mean retention time (MRT_2, h) of large and small particles for each cattle group.

Particle size	[1]Animal Group					SED
	1[I]	2[II]	3[III]	4[IV]	5[IV]	
Large	29.71	28.51	27.08	29.10	31.88	
						4.340
Small	25.11	24.97	22.77	23.57	25.40	

[1]Groups 1-4 are Criollo and Group 5 are Holstein x Criollo; to compare between breeds take average of groups 1-4 and compare with group 5 using SED in table and 19 d.f.

[I-IV]Animal size; for comparing means within the same size, take average of groups 4 and 5 and use SED=2.081 with d.f. =45. Otherwise use SED in table and 19 d.f.

In Table 3 the comparison of mean retention times of the large and small particle pools in the whole gastrointestinal tract is given. There was a significant ($p<0.001$)

particle size effect on MRT_T, such that on average the large particles were retained for over 8.5 h longer than the small particles in the whole tract. There was however no detectable influence of animal breed or animal size on the mean retention time of the particles in the whole tract.

Table 3. Comparison of whole tract mean retention time (MRT_T, h) of large and small particles for each cattle group.

Particle size	[1]Animal Group					SED
	1^{I}	2^{II}	3^{III}	4^{IV}	5^{IV}	
Large	54.07	49.99	50.01	54.10	56.72	4.617
Small	45.91	41.39	42.72	*44.50*	47.08	

[1]Groups 1-4 are Criollo and Group 5 are Holstein x Criollo; to compare between animal breeds take average of groups 1-4 and compare with group 5 using SED in table and 16 d.f.

[I-IV]Animal size; for comparing means within the same size, take average of groups 4 and 5 and use SED=1.570 with d.f. =45. Otherwise use SED in table and 16 d.f.

Particle buoyancy

Table 4 shows the comparison of the mean retention time of buoyant and non-buoyant particles in the fast turnover compartment (MRT_1) for each cattle group.

There was no significant difference in the mean retention time of the buoyant and non-buoyant particle fractions in the fast turnover compartment. The non-significant animal size and buoyancy and animal breed and buoyancy interactions indicated no evidence of breed and size effects in the retention of buoyant and non-buoyant particles in the fast turnover compartment.

Table 4. Comparison of the fast turnover compartment mean retention time (MRT_1, h) of buoyant and non-buoyant particles for each cattle group.

Particle buoyancy	[1]Animal Group					SED
	1^{I}	2^{II}	3^{III}	4^{IV}	5^{IV}	
Buoyant	9.61	8.42	6.89	7.86	10.05	2.008
Non-buoyant	10.33	9.10	7.86	*11.54*	9.29	

[1]Groups 1-4 are Criollo and Group 5 are Holstein x Criollo; to compare between animal breeds take average of groups 1-4 and compare with group 5 using SED in table and 25 d.f.

$^{I-IV}$Animal size; for comparing means within the same size, take average of groups 4 and 5 and use SED=1.395 with d.f. =45. Otherwise, use SED in table with 25 d.f.

There was no significant effect of buoyancy on the mean retention times of the particles in the slow turnover compartment (Table 5). Likewise, there were no noticeable effects of animal size and breed on the retention of the buoyant and non-buoyant particle pools as shown by the non-significance of buoyancy with animal size and buoyancy with animal breed interactions.

Table 5. Comparison of the slow turnover compartment mean retention time (MRT_2, h) of buoyant and non-buoyant particles for each cattle group.

Particle buoyancy	[1]Animal Group					SED
	1^{I}	2^{II}	3^{III}	4^{IV}	5^{IV}	
Buoyant	26.12	25.24	24.75	27.68	26.70	4.340
Non-buoyant	28.70	28.24	25.09	*24.99*	30.58	

[1]Groups 1-4 are Criollo and Group 5 are Holstein x Criollo; to compare between breeds take average of groups 1-4 and compare with group 5 using SED in table with 19 d.f.

[I-IV]Animal size; for comparing means within the same size, take average of groups 4 and 5 and use SED=2.081 with d.f. =45. Otherwise, use SED in table with 19 d.f.

Table 6 lists the mean retention times of buoyant and non-buoyant particles in the whole gastrointestinal tract. The non-buoyant particles were retained on average 2 h longer (p<0.05) than their buoyant counterparts. However, there were no significant differences in MRT_T of the buoyant and non-buoyant particle pools for animal sizes 1, 3 and 4. There were however no significant buoyancy and animal size or buoyancy and animal breed interactions detected.

Table 6. Comparison of whole tract mean retention time (MRT_T, h) of buoyant and non-buoyant particles for each cattle group.

Particle buoyancy	[1]Animal Group					SED
	1^{I}	2^{II}	3^{III}	4^{IV}	5^{IV}	
Buoyant	48.65	43.87	45.89	49.48	50.21	
						4.617
Non-buoyant	51.33	47.52	46.84	49.12	53.59	

[1]Groups 1-4 are Criollo and Group 5 are Holstein x Criollo; to compare between breeds take average of groups 1-4 and compare with group 5 using SED in table and 16 d.f.

[I-IV]Animal size; for comparing means within the same size, take average of groups 4 and 5 and use SED=1.570 with d.f. =45. Otherwise, use SED in table with 16 d.f.

Intake and gutfill

Gutfill estimates (dose/initial marker concentration), which were also corrected for alkane losses in the gastrointestinal tract using the recovery rates of C_{26}, C_{28}, C_{30} and C_{32} published by Mayes et al. (1988), [the recovery rate for C_{26} and C_{30} were obtained by interpolation] differed when model parameters for each of the four particle pools were used (Table 7). This is probably due to the different initial marker concentration estimates derived by fitting the G1G2 to the faecal excretion curves.

The results of the comparison of dry matter intake, gutfill and gutfill as a percentage of body weight between the five cattle groups, the four sizes of Criollo (groups 1 to

4) and the mature Criollo and Holstein cross (group 5) are presented in Table 7. As expected dry matter intake increased with animal size from group 1 to group 4, but there was no significant difference between the fully grown pure-bred Criollo and crossbred bulls, although the crossbred, which weighed on average more than the pure bred Criollos, consumed numerically slightly more dry matter than the their pure-bred counterparts.

Gutfill, regardless of particle pool parameters used, followed the same pattern as intake and increased with animal size within the Criollo breed, but there was no significant difference between the crossbred and the pure-bred bulls. The numerical dry matter intake advantage of the crossbred was also reflected in its numerically higher gutfill content. Although the parameters of the four particle pools yielded different gutfill estimates from one another for the same animal group, they all gave gutfill estimates that increased with increasing animals size and reflected no significant difference between the mature Criollo and Criollo and Holstein cross. The parameters of the LB pool yielded the highest estimates while those of the SD pool gave the lowest estimates, with those of the SB and LD giving estimates that were closer together and closer to those yielded by using the parameters of SD.

Gutfill expressed as a percentage of animal body weight was largely not significantly different across all animal sizes within the Criollo breed and between the Criollo and the Criollo and Holstein cross, regardless of particle pool parameter used. However for group 1 the SB particle pool parameters yielded gutfill expressed as a percentage of animal weight that was significantly lower ($p<0.05$) than that for group 2, but not significantly different from the rest of the other groups, including the crossbred bulls. Gutfill expressed as a percentage of body weight for group 2 was significantly higher than that for group 1, but was not significantly different from that of groups 3, 4 and 5.

Table 7. Comparison of dry matter intake, gutfill[j] and gutfill[j] as a percentage of body weight between the five cattle goups[k]

		Animal group					SED (d.f.)
		1	2	3	4	5	
Weight (kg)		88^{a}	146^{b}	310^{c}	446^{d}	438^{d}	15.5 (15)
Intake (g DM)		1102^{a}	2165^{b}	5784^{c}	6877^{d}	6916^{d}	340.3 (15)
Gutfill (g DM)	LB	993^{a}	1946^{b}	3738^{c}	5001^{d}	4931^{d}	336.6 (41)
	SB	473^{a}	1171^{b}	2172^{c}	2632^{d}	2823^{d}	
	LD	415^{a}	975^{b}	1931^{c}	2441^{d}	2841^{d}	
	SD	359^{a}	871^{b}	1655^{c}	1944^{d}	2108^{d}	
Gutfill as % of body weight	LB	1.15^{a}	1.33^{a}	1.21^{a}	1.12^{a}	1.13^{a}	0.118 (31)
	SB	0.54^{a}	0.79^{b}	0.71^{ab}	0.59^{ab}	0.65^{ab}	
	LD	0.48^{a}	0.67^{a}	0.63^{a}	0.55^{a}	0.66^{a}	
	SD	0.41^{a}	0.59^{a}	0.54^{a}	0.44^{a}	0.49^{a}	

[j] Gutfill=dose/initial marker concentration

[k]groups 1 to 4 are pure bred Criollo and group 5 are Criollo X Holstein cross
LB, SB, LD and SD are, respectively, large buoyant, small buoyant, large dense and small dense particle pools

Means with different superscript letters within the same row are significantly different

Discussion

Particle size

The results of the experiment presented here suggest that the large particles were retained longer than small particles in the fast turnover compartment (Table 1). This is in agreement with the general contention that large particles have a lower probability of leaving the reticulorumen than small particles (Kaske and Engelhardt, 1990; Welch, 1982; Kennedy, 1995; Illius and Gordon, 1991). The results are also in support of the proposed conceptual model (Chapter 5, Figure 1) that in the rumen there are time-dependent processes to which ingested feed residues are subjected to progressively obtain the requisite physical properties, including size reduction, for escape from the rumen through the reticulo-omasal orifice (Wylie et al., 2000). Therefore the difference in MRT_1 between the large and small particle pools can be attributed to mainly the time it took to comminute the large particles to the size of the small particles.

Lalles et al. (1991) also fitted the G1G2 model used in the present study to faecal data of early-weaned calves dosed with two small particle fractions, ytterbium-labelled indigestible residues from hay and cerium-labelled indigestible residues from a concentrate. They obtained MRT_1 (which they called MRT_2) of 8.6 h for the ytterbium-labelled indigestible residues and 7.5 h for the cerium-labelled indigestible residues. The MRT_1 estimates of both indigestible residues are longer than the average MRT_1 of 6.81 h for the small particles found in this study. However, the MRT_1 average of 6.81 h reflects retention times of both fermented and non-fermented particles, and when only the fermented particles, which are comparable to the indigestible residues, are taken into account the MRT1 of small particles is 7.63 h, which is comparable to the estimates reported by the above workers.

Mean retention time in the fast turnover compartment did not seem to be influenced by animal size or breed (Table 1). These results can be interpreted to mean that the time-dependent processes to which ingested feed residues are subjected in the rumen to progressively obtain the requisite physical properties for escape from the rumen was similar in length for the cattle sizes and genotypes used in this study. It may be argued the wetting of feed residues and their colonisation by microbes in the rumen, processes that indirectly contribute to particle size reduction, may take place at similar speeds irrespective of animal size. However, chewing efficiency and thus size reduction efficiency was expected to favour the more mature animals, but this difference in chewing efficiency was probably masked by the difference in the feed quantities the animals consumed. In other words, even though the younger bulls may not have been as efficient as their more mature counterparts in reducing the particle size of ingested feed residues, they did not eat as much and therefore did not process as much as the older bulls, which may have not have shown the superior chewing efficiency of the older bulls.

The mean retention times of the large particles reported here are 7.5 h shorter than that indicated by Ellis et al. (1994) who used the G1G2 model to estimate MRT_1 in a steer fed bermudagrass hay. The difference between the results of this study and those reported by Ellis et al. (1994) could be attributed to the differential passage rates expected of diets with different physical and chemical compositions because the size and duration of the raft that forms in the rumen are influenced by feed type (Ehle and Stern, 1984; Faichney, 1993). On the one hand, the sub-tropical bermudagrass hay promoted the formation of a large digesta raft that could have trapped particle and thus slowed down their rate of passage. On the other hand, the cattle used in this experiment grazed a Brachiaria decumbens sward and therefore the rafts formed in their rumens were probably not as large as the raft that formed in the steer fed on hay, which Ellis et al. (1994) found to constitute approximately 90% of the mass of digesta in the rumen. By contrast, Pasha et al. (1994) observed a 7 h longer mean particulate retention time for high moisture forage than for hay in sheep.

The mean retention time of the small particles in the slow turnover compartment was on average shorter than that of the large particles (Table 2). Such results are in contradiction of the proposed model assumption that in this compartment all feed particles have been reduced to a size small enough escape through the reticulo-omasal orifice and therefore the mean retention time of the particle fractions labelled large and small should be similar. In the slow turnover compartment the escape of particles follows the pattern of slow turnover dilution and the chances of escape for all particles in this compartment are equal (Ellis et al., 1994). It should however be taken into consideration that although the slow turnover compartment and the fast turnover compartment are modelled as two entities they are physically not separate but represent two steps to which feed residues are subjected in the reticulorumen during the digestion process. Also, the physical separation of these two compartments is influenced by diet and interval between meals (Ellis et al., 1994).

Sutherland (1988) identified interactions between mixing actions caused by rumen motility and unmixing actions of forage particles, processes that help to modulate the escape from or retention in the rumen of particles. This highlights the complexity of the rumen and that other factors besides particle size may have been involved in the sorting mechanisms. For instance, it is possible that the entrapment of the large particle fraction in the raft that formed within the rumen was so pronounced that by the time the large particles had attained eligibility for escape appreciable amounts of the small fraction (which because of size was trapped to a lesser extent) had escaped out of the rumen. Kennedy and Doyle (1993) suggested that there is very little entrapment of small particles in the rumen raft. Furthermore, large particles because of greater rate of production of fermentation and their shape which requires less gas per unit of digesta to remain buoyant (Sutherland, 1988) may have remained much longer than the small particles in the rumination pool. In such circumstances size differences within the first (fast turnover) compartment would influence the course of events and not clearly reveal the reduced or eliminated size difference in the second compartment in terms of mean retention time. Kennedy and Doyle (1993) postulated that separation of particles from the rumen raft would be more difficult if the raft was formed from the consumption of tropical grasses, as was the case in this study, because of their relatively rough surfaces. It has also been observed that the rates of escape of eligible particles in terms of size may vary widely (Murphy and Kennedy, 1993).

However the results of this study are consistent with the differential mean retention times of large and small particles experienced in other studies, although most did not attempt to resolve the mean retention time in the rumen into time spent in a particle reduction pool (not immediately associated with escape) and time spent in a small particle pool eligible for escape. Welch and Smith (1978) showed that polypropylene ribbons of 1.5, 1.0. 0.5 cm in length passed through the reticulo-omasal orifice of cattle in larger quantities than those of 2 cm in length. Kennedy (1995) reported an inverse curvilinear relationship between particle ranging in size from 50 to 4750 μm and rate of passage from the reticulorumen of swamp buffaloes and cattle fed on rice straw and various energy and protein supplements.

The mean retention times in the slow turnover compartment of 29.25 and 24.37 h for the large and small particles, respectively, reported here are comparable to the estimate of 27 h for ytterbium-labelled indigestible hay residues found by Lallès et al. (1991). The MRT_2 of the small particle fraction used in this study and in the study conducted by Lallès et al. (1991) are not expected to differ much as they were similar in size. However, the different particle preparation processes and the different diets used in the two experiments were probably responsible for the discrepancy.

Longer mean retention time of large particles compared to small in the whole gastrointestinal of ruminants has been reported by several investigators with nylon particles (Kaske and Engelhardt, 1990; Kaske et al., 1992)) and hay (Bernard et al., 1998). Murphy et al. (1989) observed higher rumination and lower passage rates for long plastic particles, particularly those longer than 5mm, compared to short particles in swamp buffaloes and cattle fed mainly on a rice straw diet. The results of this experiment that particles ground to pass a 6 mm screen were retained longer than those ground through a 1mm support those findings. The difference in the passage rates and thus mean retention times of large and small particles is due to the extra time needed to comminute large particles to small particles, but the rate at which this reduction is done can be influenced by particle physical properties (Kennedy and Doyle, 1993). This view and the result of a longer mean retention time of the large particle fraction in the whole tract are in concurrence with the model proposal outlined in Chapter 4 of this manuscript that large particles have to be reduced to a certain degree of fineness before they can pass through the gut in relatively large quantities.

Buoyancy

Although there seems to be a general consensus among researchers that digesta particles have to be reduced in size to be able to flow out of the reticulorumen into the post-ruminal tract, size reduction on its own does not guarantee passage through the reticulo-omasal orifice. Welch (1982) reported that more than half the dry matter in the rumen of hay-fed steers could pass a 600 μm sieve, but could not be cleared rapidly from the rumen. Data from experiments with cattle (Evans et al., 1973) and sheep and cattle (Poppi et al., 1981) have shown that most of the particulate matter in the reticulorumen was smaller than the reticulo-omasal orifice and that the reticulo-omasal orifice seemed large enough to accommodate particles larger than the ones found in the faeces of ruminants (Kennedy and Doyle, 1993; McBride et al., 1984). Thus there seems to be another characteristic or mechanism, in addition to size, by which particles are sorted in the reticulorumen for onward flow.

Studies with plastic particles have indicated that density is involved in particle selection and that particles of density of about 1.2 g/ml (King and Moore, 1957; desBordes and Welch, 1984) or 1.34 g/ml (Murphy et al., 1989) have the shortest mean retention time in the rumen and in the gut. The results of this experiment however did not indicate a significant effect of buoyancy in the slow and fast turnover compartments. Such results were unexpected as some particles were prefermented in rumen liquor to increase their density and thus their rate of passage from the rumen. However, lack of evidence of the influence of density on passage has been reported by others. For instance, Kennedy (1995) could not establish a connection between the specific gravity of particles, measured as particle sedimentation rate in the reticulum, and their probability of passage from the rumen. Neel et al. (1995) increased the specific gravity of dried forages by reconstituting with water, but this did not increase the rate of passage of their digesta from the rumen of crossbred steers; reconstitution decreased the passage rates for timothy hay and had no demonstrable effect on the rates of passage of alfalfa.

The effect of buoyancy was demonstrated on the particle mean retention times in the whole gastrointestinal tract in that the non-buoyant particles were retained longer than the buoyant particles. However, buoyancy as envisaged in the conceptual model of this study was expected to display the opposite of the effect suggested by the results of this study, i.e. the fermented particles were expected to have a shorter MRT_T than the non-fermented particles. These results are difficult to explain in terms of difference in specific gravity between the fermented and the non-fermented fractions because the specific gravities of the two particle fractions were not measured. However, other experimental findings would suggest that they may have reached a maximum specific gravity of about 1.4g/ml (Hooper and Welch, 1985) or 1.5 g/ml (Evans et al., 1973) in rumen liquor. The rate at which prefermented and non-fermented particles rehydrated, which would influence their rate of density increase, on entering the reticulorumen would have determined their rate of passage from the gut. During preparation of the particles, the prefermented particles tended to be fluffier, which suggested a higher bulk dry volume, than their non-fermented counterparts. Ehle and Stern (1984) stated that feedstuffs with a higher volume per unit mass have a lower rate of passage than smaller bulk volume feedstuffs. This may at least partly explain the lower retention of the non-fermented compared with fermented particles in the digestive tract.

Fermentation in rumen liquor should have depleted the digestible material in the non-buoyant fractions and therefore the buoyant and non-buoyant fractions should have been chemically dissimilar. The capacity of forages to absorb water and increase in density is related to their chemical characteristics (Martz and Belyea, 1986). Forages with a high content of structural material like cellulose and lignin are expected to hydrate slower than high quality forages (Martz and Belyea, 1986). It is therefore plausible that the fermented material used in this study resisted hydration and thus density increase because lignin, which has a low affinity for water, was the predominant material left in them after fermentation in rumen liquor. Consequently, the non-fermented fractions may have rehydrated, increased in density and passed faster than the fermented particles.

Alternatively and as previously stated, the purpose of incubating the non-buoyant pools in rumen liquor was to increase their specific gravity so as to facilitate their movement along the digestive tract. If this aim were indeed achieved, it would appear that the incubation increased their density beyond the optimum density range for passage such that on entering the reticulorumen they quickly sedimented to the reticulorumen floor, where their movement was retarded. Furthermore, fermentation-based buoyancy in the rumen was probably not maintained for long in the prefermented particles because they were depleted of most fermentable carbohydrates during the prefermentation process, which would have speeded their sedimentation rate. The results of Hooper and Welch (1985) and Evans et al. (1973) suggested that the functional specific gravity of the fermented hay would not have reached much higher than 1.3 and 1.5. If this was the case and their rate of density increase was faster than those of the non-fermented pool, they would not have benefited from fermentative buoyancy, which would have enabled them to be selected for ruminative chewing and deposition into a reticulorumen position favourable for onward passage. As previously stated, this effect (reduced ability to escape) would have been more pronounced on the large particle fraction.

There was no evidence of the effect of animal size on the mean retention times of the particles in the fast turnover and slow turnover compartment and in the whole gastrointestinal tract from the collected data. This was unexpected as intake and digesta passage rates have been shown to be a function of animal size, at least across species, in the intake prediction model of Illius and Gordon (1991), which uses rumen kinetics integrating animal size and plant characteristics. However, the results of this study are consistent with the findings of Lalles et al. (1991) who measured mean retention times of dietary residues in the stomach and the whole tract of early-weaned dairy calves. They reported a mean retention time of 46.5 h, using the G2G1 model used in this study, for hay residues. This figure is slightly lower than, but of the same order as the average of the mean retention times of the buoyant and non-buoyant fractions (47.62 and 49.68 h, respectively) in the whole tract of the cattle used here. It is also well within the range of the lowest and highest mean retention times of the particles of all the cattle groups used here, which would suggest that mean retention times within the weight range used in this study are not influenced by animal size. This is in contrast to the observation made by Faichney (1993) who stated that reticulorumen mean retention time tends to be lower in young ruminants.

Intake and gutfill

The dry matter intake figures for groups 1 and 2, particularly group 1 may not reflect the true dry matter intake capacity for those bulls because, even though they were mainly grazing *Brachiaria decumbens* in a 2 ha plot away from their mothers, they were allowed to suckle for about 1h after milking in the morning and in the afternoon. There are limited data on dry matter intake by young bulls, but according to Ensminger and Olentine (1978) young bulls should get a feed allowance that is between 2 and 2.25% of their liveweight. The dry matter intake estimates for the group 1 bulls are lower than this recommendation, but do not include the amount of milk they consumed in the morning and afternoon after milking. Also, ruminants as young as those in groups 1 and 2 cannot consume large amounts of grass, particularly tropical grasses which tend to be coarser than their temperate counterparts. Groups 3, 4 and 5 consumed grass dry matter in excess of 1.5% of their weight, which is within the recommended allowance range of between 1.5 and 3% of body weight for mature bulls, depending on animal condition and individuality (Ensminger and Olentine, 1978).

Gutfill increased significantly ($p<0.05$) with animal size, but not between the mature Criollo and Criollo and Holstein cross (Table 7). This increase was probably due to the dry matter intake increase, which also increased proportionately according to animal size. This trend is in agreement with the results of Pond et al. (1984) who also reported a 67% postpartum gastrointestinal tract fill increase with intake compared to prepartum in ewes fed on Coastal bermudagrass pellets ad libitum. Pond et al. (1984) however used Co-EDTA to measure gutfill.

It is difficult to forward a reason for the different estimates for gutfill and gutfill expressed as a percentage of body weight yielded derived by using parameters from the different particle pools. This is probably due to the different initial marker concentration estimates derived by fitting the G1G2 to the faecal excretion curves. Theoretically, where there is perfect and instantaneous mixing of the introduced marker with resident digesta in the rumen, the initial marker concentration estimates should be similar regardless of particle pool parameters used, provided the particle pool markers were introduced into the rumen at the same time or within a short space of time, as was the case in this study. However, because the four particle pools were different in physical and chemical attributes (due to the different treatments to which they were subjected before coating with alkanes) their mixing capabilities would be expected to be different and would display varying degrees of deviation from the ideal scenario of perfect and instantaneous mixing.

Worrel et al. (1986) also reported different gastrointestinal tract fill estimates for forage particles differing in size from 850 to more than 1680 microns. The parameters from the smallest particle fraction (850 microns) yielded the lowest gastrointestinal tract fill expressed as a percentage of body weight estimate, which was comparable to the estimates yielded by using the parameters from SB, SD, LD particle pools in this study for. The estimates derived from using the parameters of the LB are of the same order of magnitude as those of the particle fraction greater than 1680 microns in the study reported by Worrell et al. (1986).

Conclusion

The large particle fraction had a longer retention time than the small particle fraction in the fast turnover compartment, in the slow turnover compartment and in the whole gastrointestinal tract is in support of one aspects of the proposed model that food particles have to be reduced to a small size in order to increase their rate of passage out of the rumen. However, the differential passage rates of the small and large particles out of the slow turnover compartment raises questions about the assumptions of the model that particles in this compartment are eligible, in terms of size, for passage out of the rumen and that their escape can be described by mass action competition for escape. If the model assumptions held true in this experiment the mean retention times of the large and small particles would have been similar because after reduction in the slow turnover compartment they would have had equal chances of escape out of the reticulorumen, unless the rate of escape of the small particles out of the reticulorumen was slower than the rate of reduction of the large particles. Therefore on the bases of size the experimental results did not lend credence to the assumptions of the model in the slow turnover compartment, which points out the existence of other forces which also play a role in the sorting of particles for escape out of or retention in the rumen.

Buoyancy, another physical factor that was proposed in the conceptual model and widely thought to be involved in the selective retention in or passage out of the rumen could not be demonstrated by the results of this experiment because there was no evidence of the involvement of particle buoyancy in both the slow and fast turnover compartments. However, the dense particles were held longer than the buoyant particles in the whole gastrointestinal tract, which points to the possibility of buoyancy-employing particle sorting mechanism somewhere in the gastrointestinal tract. Therefore, although the conceptual model was proper in its assumption of the involvement of buoyancy in the passage of particles through the gastrointestinal tract, the experimental results could not demonstrate it was solely the rumen.

The results of the retention of particles in animals of the four different sizes and two genotypes lead to the conclusion that retention time in the rumen and the whole tract was not influenced by size or genotype. Gutfill expressed as a percentage of body weight also did not change according to animal size or genotype. However, amount of gutfill and intake increased with animal size, but showed no significant difference between the purebred Criollo and the Criollo and Holstein cross.

CHAPTER 7

General discussion

Intake

The main thrust of this research effort was to evaluate the use of alkanes as markers for estimating dry matter intake (DMI) and diet selection by ruminants in two studies. The first study, which was carried out indoors, sought to investigate the feasibility of using alkanes to estimate DMI and botanical composition of the consumed diet when animals are given diet mixtures, to assess the consequences of diurnal variation in alkane concentration in the gut on the accuracy of the n-alkane technique and to test the efficacy of dosing once daily, as opposed to twice daily, with an even-chain alkane. To that end, three experiments, 1, 2 and 3, were conducted. Experiment 1 used lambs at 30 and 45% of projected mature weights to compare estimates derived from the n-alkane method with direct measurements of a) grass dry matter intake when pelleted grass was offered alone, b) lucerne dry matter intake when pelleted lucerne was offered alone, and c) the proportion and dry matter intake of lucerne and grass in selected diet when pelleted grass and pelleted lucerne were offered together as a choice. Experiment 2 was designed to investigate the diurnal pattern of excretion of the faecal n-alkane ratios of tritriacontane (C_{33}) to dotriacontane (C_{32}) over a 24-h period when lambs were fed *ad libitum* or a restricted amount of feed. Experiment 3 sought to compare faecal alkane ratios of C_{33} to C_{32} between lambs dosed once daily and twice daily with e C_{32}.

The results of experiment 1 generally indicated a close agreement between the observed and the estimated dry matter intake of both lucerne and grass at 30 and 45% mature sizes when given singly, as evidenced by the fitted regression lines describing the relationships between the observed and estimated quantities not being significantly different from the lines of equality [i.e. the slopes and the intercepts were not significantly different from one and zero, respectively (Table 3)].

However, at 30% mature size, for the lambs fed on grass only, dry matter intake was slightly overestimated and for those fed on lucerne and grass as a choice, the dry matter intake of lucerne was underestimated by the alkane technique. The discrepancy between the observed and estimated figures at 30% mature size could be due to experimental error in the alkane technique or measurement error in the compilation of the observed quantities. The observed intake amounts are also subject to a degree of measurement error, which can influence the agreement between the observed and estimated values. Furthermore, at 30% mature size the lambs were still adapting to the experimental conditions and possibly some of food recorded as consumed may include pellets that spilled through the slats of the floor. A conclusion than can be drawn from the general agreement between the observed and the measured quantities is that the alkane technique can be used to estimate the dry matter intake of some forages by ruminants. Another advantage of the alkane technique over other methods of estimating DMI is that it gives DMI estimates that reflect animal individuality, as opposed to using digestibility estimates based on in vitro digestibility or other animal which may have different digestion ability from that of the animal whose intake is estimated. This attribute makes the method suitable for estimating intake for animals with different physiological status, like pregnant or lactating animals or during cold weather when the intake of animals may be higher than that of the animal used to estimate digestibility (Dove, et al., 2000; Young, 1983).

The diet composition estimate upon which subsequent estimates of the quantities of individual components of a mixed diet is derived from solving simultaneous equations. For the equations to be solvable, the number of n-alkanes whose concentrations are used in the equations should ideally be equal to the number of the individual components of the diet, although a solution can be obtained by using one alkane less than the number of diet components (Dove and Mayes, 1991). In most situations 4 or 5 alkanes can be used because others are found in such small quantities that it is difficult to detect them using the available chromatographic technology. Thus, no more diet components than the number of detectable alkane concentrations can be resolved from a mixture, which means four or five diets, until highly sensitive equipment is available. In many situations ruminants utilise grazing or browsing material with a wider variety than the technique can resolve into components, which is a handicap of the technique. Therefore another compound or compounds of plants which can provide finger print identity for each plant species, which can be employed using the same principle of solving simultaneous equations would increase the scope of using simultaneous equations to estimate diet selection.

Furthermore when the simultaneous equations method is used to resolve a diet with only two components, in which case the concentrations of two n-alkanes should be chosen and used in the simultaneous equations, it is not always obvious which two to choose from several possibilities (Figure 6 of chapter 3). Different diet selection estimates can be derived from different pairs of alkane concentrations used. Theoretically the technique would be unreliable in estimating diet selection when the alkane concentration patterns are not markedly different and the total alkane contents are not similar between the diets on offer.

The different estimates of diet selection derived from using different recovery rates highlights the sensitivity of the technique to changes in recovery rates. It is therefore imperative that recovery rates be determined experimentally for diet selection experiment, rather than relying on the use of published figures. There is also a possibility that the percentage of recovery of alkanes is also subject to physiological status of the animal like lactation, pregnancy and cold stress. Knowledge of the extent of individual variability in recovery rates would strengthen the technique.

The study also sought to investigate diurnal variation in the concentrations of the dosed and natural alkane, C_{32} and C_{33}, respectively, used in the formula for estimating dry matter intake because any significant diurnal variation would mean that dry matter intake estimates for the same animal would vary depending on the collection time of the faecal samples. To the credit of the alkane technique the ratio of the concentration of C_{33}:C_{32} in the faeces showed no appreciable diurnal variation irrespective of whether the lambs were restricted or *ad libitum* fed. Thus unbiased intake estimation can be made using faecal samples collected any time of the day after a steady state had been established and feeding level would not influence the accuracy of the estimate.

Dosing once daily, compared to twice daily, would have the practical advantage of reducing animal handling and minimising invasion. That would also increase grazing time and thus intake, especially in extensive grazing systems. However bigger within day variations in the ratios of natural to dosed alkanes in the faeces when animals are dosed once daily than when they are dosed twice daily has been reported. Although the results of this study could not conclusively prove the feasibility of dosing once daily, there were no differences in the ratios of the concentrations of C_{33}:C_{32} between the two dosing strategies until the fifth day of dosing (Table 4). A possible cause of this difference may have been that some of the lambs were dosed once daily were inadvertently fed on lucerne which has a lower concentration of C_{33} than grass, which would affect ratios of the concentrations of C_{33}:C_{32}. For any meaningful conclusion to be drawn from a study comparing the two dosing strategies, the same diet should have been fed to all the lambs in both groups and this not having been the case in this study the efficacy of dosing once daily could not be fairly evaluated.

Mayes et al (1986) compared the feasibility of using different odd (dosed) and even (natural) alkane pair combinations and found that most pairings used slightly underestimated actual intake, except the C_{32} dosed-C_{33} natural alkane pair, which gave an estimate that equalled the measured intake. The pair of C_{32} and C_{33} works well for most temperate grass species because they have adequate quantities of C_{33}, but tropical species sometimes do not contain enough quantities of C_{33} to make pairing with C_{32} a feasible option. In some situations C_{32} may be prohibitively expensive or unavailable, as was the case when the second study was conducted. In such cases therefore a need exists to explore other alkane pair combinations.

The second study thus used the alkane pair of C_{36} and C_{35} as the dosed and natural alkanes, respectively, to compare the voluntary dry matter intake of *Brachiaria decumbens* by dry and lactating Criollo cows during the wet season and dry and lactating cows during the dry season of Bolivia. The choice of this alkane pair was based on the assumption that they are adjacent and therefore would have comparable recovery rates, a requirement for accurate estimation of DMI by the alkane method. A criticism for choosing C_{35} would be that sometimes there are not enough quantities of it in tropical grass species and would therefore be difficult to quantify. Casson et al. (1990) cited by Laredo et al. (1991) recommended that the concentration of the odd chain alkane used in the method be more than 50 mg/kg of herbage DM. This does not seem to be a valid criticism for the study reported here because the equipment used had no problems with quantifying the concentrations of C_{35} during the dry season and during the wet season when they dropped below 50mg/kg DM. There is no reason to doubt the accuracy of the DMI estimates on the ground of inadequate amounts of C_{35} because the same equipment was used to extract and quantify herbage alkane concentration lower (lucerne C_{33} = 17.4 mg/kg DM) than the suggested minimum threshold of 50mg/kg DM and obtained intake estimates which closely agreed with the observed intake of sheep (see Table 1 of Chapter 3 of this manuscript).

The alkane method using the pair of C_{35} and C_{36} has helped to achieve the aim of the second study- to compare voluntary intake of *Brachiaria decumbens* by dry and lactating cows during the wet and the dry seasons. Because there was no parallel cross validation experiment of direct measurements conducted with study II, conclusive claims of the accuracy of using C_{36} and C_{35} cannot be made. More investigations into the accuracy of using C_{36} and C_{35} to estimate intake are warranted, which should be validated by direct measurements. However, it can be stated that the alkane pair combination can be used to estimate DMI of tropical forages by cows with at least reasonable accuracy, particularly when the availability of other alkanes like C_{32} is a problem.

Passage rates

The use of alkanes as markers in passage rates studies has a number of advantages over some of the most popular markers. For instance, the results of the gas production experiment carried out at the beginning of the third study suggested that alkanes can be attached to fibre particles without altering the fermentation characteristics of the fibre to which they are attached. The same cannot be said of most other markers. Chromium reduces digestibility and increase the specific gravity of the particles to which it is attached. Rare earths can also reduce digestibility, depending on method of attachment and can react with proteins and carbohydrates, forming insoluble complexes which can move independently of the material to which rare earths are attached. Another advantage about using alkanes as markers is that several types are available and several particle pools can be marked, dosed and their passage rates monitored at the same time.

Passage rates experiments, Studies III and IV, sought to validate a conceptual model (Figure 1 of chapter 5) which proposed the involvement of buoyancy and size in the rate of passage of particulate digesta from the rumen. The model proposed that eligibility of digesta particles for passage out of the rumen is a function of the length of time they have spent in the rumen because particle size reduction and density increase are time-dependent processes.

In study IV, four particle pools of 2 sizes and 2 densities in a factorial manner were labelled with 4 different alkanes, dosed to sheep and their rates of passage in the digestive tract were compared. Study IV sought to compare the rates of passage of the four particle pools as used in study III in the gastrointestinal tracts of four sizes and two genotypes of cattle. The four particle pools were small and dense (SD), small and buoyant (SB), large and dense (LD) and large and buoyant (LB). Therefore, according to the proposed model, the smallest and densest particles (SD) would have the fastest and the largest and most buoyant (LB) the slowest rates of passage out of the rumen in the digestive tract. The large, dense (LD) and the small, buoyant fractions (SB) would have somewhat intermediate rates of passage.

The results of the two studies did not fully validate the proposed model: in support of the model, while the small particles had a shorter retention time than the large ones in the rumen and in the whole tract, contrary to model prediction, the particles labelled dense had a longer retention time than the particles thought to be buoyant. That result brings out the involvement of both particle size and particle buoyancy in the mechanism for sorting particles for passage out of or retention in the rumen, but not in the manner proposed by the model. Incubating fibre particles in rumen liquor increases their density, but incubating them for as long as was done in these studies, may have made them too dense to readily migrate out of the reticulorumen ahead of the non-fermented particles. Therefore the long incubation made them denser than the optimum density range for passage out of the rumen and their passage was retarded so much so that the non-fermented particles passed more rapidly out of the rumen and in the whole tract.

The lesson learnt from the passage rates studies is that the specific gravities of all four particle pools should have been measured before dosing to the animals to determine if fermenting them for long indeed made them too dense for rapid passage out of the rumen and would have made rate of passage comparisons between the particle pools more convincing. Further research on particle kinetics in the digestive tract should include fermenting particles of different size for different lengths of time and quantifying their densities and extent of digestion before measuring their rates of passage. That would also provide quantitative support for relating digestion (and fermentation-based buoyancy), particle size and density to rates of passage.

One of the objectives of the fourth study was to investigate how intake-related attributes like rumen fill and passage rates of particles change in relation to changes in body mass as animals grow and to determine if these attributes would be different between animals of different breeds. The similarity of the retention time of each of the four particle pools in the cattle of the four different sizes and two genotypes (pure-bred Criollo and Criollo/Holstein cross) lead to the conclusion that retention time in the rumen and the whole tract was not influenced by size or genotype. However, the two cattle breeds were not genetically distant and therefore it can be argued that they would have more common traits (including rates of passage) than animals that are genotypically dissimilar. More convincing evidence of similarity or otherwise of rates of passage could be derived from experiments making use of distantly related genetic stock like *Bos taurus* and *Bos indicus*. It would be interesting to find out if the superiority of tropical cattle breeds over their temperate counterparts in utilising poor quality tropical forages would have a basis of a physiological adaptation mechanism of digesta processing, like slower rates of passage, that temperate breeds do not have.

Gutfill increased with animal size according to DMI, but showed no significant difference between the pure-bred Criollo and the Criollo and Holstein cross. Gutfill expressed as a percentage of body weight also did not change according to animal size or genotype, which leads to the conclusion that gutfill remains a constant proportion of body wait throughout the growth and development of the animal. One flaw with this conclusion is that the gutfill content and gutfill content expressed as a percentage of body weight were derived mathematically from the parameters generated after fitting the G1G2 model to the faecal excretion data. Thus any inaccuracies or experimental errors in the fitting procedure and interpretation of the generated parameters will be passed on to the gutfill estimates. Further research into changes in gutfill content and gutfill as a percentage of body weight using more direct measurements like physically emptying the rumen contents is therefore warranted.

References

Allen, M. S. 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74: 3063-3075.

Bae, D. H., J. G. Welch and A. M. Smith. 1981. Efficiency of mastication in relation to hay intake by cattle. J. Anim. Sci. 52: 1371- 1375

Barry, T. N., G. J. Faichney and C. Redekopp. 1985. Gastrointestinal tract function with somatostatin. Aust. J. Biol. Sci. 38: 393-403.

Baumont, N., N. Seguier and J. P. Dulphy. 1990. Rumen fill, forage palatability and alimentary behaviour in sheep. J. Agic. Sci. 115: 277-284.

Bernard, L., J. P. Chaise, E. Delval and C. Poncet. 1998. Validation of the main modelling methods for the estimation of marker retention times in the different compartments of the gastrointestinal tract in sheep. J. Anim. Sci. 76: 2485-2495.

Blaxter, K. L. N. McC. Graham and F. W. Wainman. 1956. Some observations on the digestibility of food by sheep, and on related problems. Br. J. Nutr. 10: 69-91.

Blümmel, M. and E. R. Ørskov. 1993. Comparison of in vitro gas production and nylon bag degradability of roughage in predicting feed intake in cattle. Anim. Feed Sci. & Technol. 40: 109-119.

Burns, J. C., K. R. Pond and D. S. Fisher. Measurement of forage intake. 1994. *In*: J. C. Fahey et al., eds. Forage Quality, Evaluation and Utilization. Crop Science Society of America. Lincoln, Nebraska. p. 494-532.

Campling, R. C. and M. Freer. 1962. The effect of specific gravity and size on the mean time of retention of inert particles in the alimentary tract of the cow. Br. J. Nutr. 16: 507-518.

Campling, R. C., M. Freer and C. C. Balch. 1961. Factors affecting the voluntary intake of food by cows. 2. The relationship between the voluntary intake of roughages, the amount of digesta in the reticulorumen, and the rate of disappearance of digesta from the alimentary tract. Br. J. Nutr. 15: 531-540.

Cardoso, E. G., V. P. B Euclides and J. M. Da Silva. 1993. Stockpiling of Brachiaria decumbens for steers maintenance during the dry season. Proceedings of the XVII International Grassland Congress.

Chen, W., J. M. Scott, G. J. Blair and R. D. B. Lefroy. 1999. Using plant cuticular alkanes to study plant-animal interactions on pastures. Can. J. Anim. Sci. 79: 553-556.

Colucci, P. E., G. K. Macleod, W. L. Grovum and I. McMillan. 1984. Comparative digestion and digesta kinetics in sheep and cattle. Can. J. Anim. Sci. 64 (suppl.): 173-174.

Colucci, P. E., L. E. Chase and P. J. Van Soest. 1982. Feed intake, apparent diet digestibility and rate of particulate passage in dairy cattle. J. Dairy Sci. 65: 1445-1456.

Dado, R. G. and M. S. Allen. 1995. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. J. Dairy Sci. 78: 118-133.

desBordes, C. K. and J. G. Welch. 1984. Influence of specific gravity on rumination and passage of indigestible particles. J. Anim. Sci. 59: 470-475.

Deswysen, A. G. and W. C. Ellis. 1988. Site and extent of neutral detergent fiber digestion, efficiency of ruminal digesta flux and fecal output as related to variations in voluntary intake and chewing behavior in heifers. J. Anim. 66:2678-2686.

Deswysen, A. G., P. A. Dutilleul and W, C. Ellis. 1989. Quantitative analysis of nycterohemeral eating and ruminating patterns in heifers with different voluntary intake and effects of monensin. J. Anim. Sci. 67: 2751-2761.

De Vega, A., J. Gasa, J. A. Guada and C. Castrillo. 2000. Frequency of feeding and form of Lucerne hay as factors affecting voluntary intake, digestibility, feeding behaviour, and marker kinetics in ewes. Austr. J. Agric. Res. 51: 801-809.

Dhanoa, M. S., R. C. Siddons, J. France and D. L. Gale. 1985. A multi-compartment model to describe marker excretion patterns in ruminant faeces. Br. J. Nutr. 49: 663-671.

Dillon, P. and Stakelum, G. 1990. Dosed and herbage alkanes for predicting silage intake with dairy cows: the effect of concentrate type and level of feeding. Proceedings of the VII European Grazing Workshop (Wageningen, The Netherlands) October 1990.

Dove, H. 1992. Using the n-alkanes of plant cuticular wax to estimate the species composition of herbage mixtures. Austr. J. Agric. Res. 43, 1711-1124.

Dove, H., J. A. Milne and R. W. Mayes. 1990. Comparison of herbage intakes derived from *in vitro*- or alkane-based digestibilities. Proc. N. Z. Soc. Animal Prod. 50: 457-459.

Dove, H. and R. W. Mayes. 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: A review. Austr. J. Agric. Res. 42: 913-952.

Dove, H. and R. W. Mayes. 1996. Plant wax components: a new approach to estimating intake and diet composition in herbivores. J. Nutr. 126: 13-26.

Dove, H. and Moore, A. D. 1995. Using a least-squares optimization procedure to estimate botanical composition based on the alkanes of plant wax. Austr. J. Agric. Res. 46: 1535-1544.

Dove, H., M. Freer and J. Z. Foot. 2000. The nutrition of grazing ewes during pregnancy and lactation: a comparison of alkane-based and chromium/*in vitro*-based estimates of herbage intake. Austr. J. Agric. Res. 51: 765-777.

Ehle, F. R. 1984. Influence of feed particle density on particulate passage from the rumen of a Holstein cow. J. Dairy Sci. 67: 693-697.

Ehle, F. R. and M. D. Stern. 1984. Physical and chemical variables influencing particle passage and size reduction. *In*: R. L. Baldwin and A. C. Bywater, eds. Proceedings of the second international workshop on modelling ruminant digestion and metabolism. University of California, Davis, 18-20 September 1984. p. 27-33.

Ehle, F. R., R. Martin and J. E. Wheaton. 1984. Influence of forage source and level on intake, milk production, growth hormone, rate of passage and body composition during lactation. Can. J. Anim. Sci. 64 (suppl.): 82-83.

Ellis, W. C., J. H. Matis and C. Lascano. 1979. Quantitating ruminal turnover. Fed. Proc. 38: 2702-2706.

Ellis, W. C., J. H. Matis, T. M. Hill and M. R. Murphy. 1994. *In*: J. C. Fahey et al., eds. Forage quality, evaluation and utilization. Crop Science Society of America. Lincoln, Nebraska. p.423-481.

Ellis, W. C., D. P. Poppi, J. H. Matis, H. Lippke, T.M. Hill and F. M. Rouquette, Jr. 1999. Dietary-digestive-metabolic interactions determining the nutritive potential of ruminant diets. *In*: H. G. Jung and G. C. Fahey, Jr., eds. Proceedings of the Vth international symposium on the nutrition of herbivores. San Antonio, Texas, 11-17 April 1999. p. 71-96.

Ensminger, M. E. and C. G. Olentine, Jr. 1978. Feeds and nutrition. Ensminger publishing Company, Clovis, California. p. 643-649.

Euclides, V. P. B., A. H. Zimmer and M. P. Oliveira. 1993. Evaluation of Brachiaria decumbens and Brachiaria brizantha under grazing. Proceedings of the XVII International Grassland Congress.

Evans, E. W., G. R. Pearce, J. Burnett and S. L. Pillinger. 1973. Changes in some physical characteristics of the digesta in the reticulo-rumen of cows fed once daily. Br. J. Nutr. 29: 357-376.

Ewing, P. V. and F. H. Smith. 1917. A study of the rate of passage of food residues through the steer and its influence on digestion coefficients. J. Agric. Res. 10: 55-63.

Fahey, G. C. and H. G. Jung. 1983. Lignin as a marker in digestion studies: a review. J. Anim. Sci. 57: 220-225.

Faichney, G. J. 1983. The effect of physical form of lucerne hay on the passage of markers through the rumen of sheep. Proceedings of the Nutrition Society of Australia 8: 186.

Faichney, G. J. 1986. The kinetics of particulate matter in the rumen. *In*: L. P.Milligan, W.L. Grovum and A. Dobson, eds. Control of Digestion Metabolism in Ruminants. Englewood Cliffs, New Jersey: Reston. p. 173-195.

Faichney, G. J. 1993. Digesta flow. *In*: J. M. Forbes and J. France, eds. Quantitative Aspects of Ruminant Digestion and Metabolism. CAB International, Oxon. p.53-85.

Faichney, G. J. and D. A. Griffiths. 1978. Behaviour of solute and particle markers in the stomach of sheep given a concentrate diet. Br. J. Nutr. 40: 71-80.

Faichney, G. J., C. Poncet and R. C. Boston. 1989. Passage of internal and external markers of particulate matter through the rumen of sheep. Repro. Nutr. Devel. 29: 325-337.

Forbes, J, M. 1993. Voluntary feed intake. *In*: J. M. Forbes and J. France, eds. Quantitative Aspects of Ruminant Digestion and Metabolism. CAB International, Oxon. p.479-494.

Forbes, J. M. 1986. The Voluntary Food Intake of Farm Animals. Butterworths, London.

Forbes, J. M. 1995. Voluntary Food Intake and Diet Selection in Farm Animals. CAB International, Oxon.

Forwood, J. R., P. Stypinski, T. Mawhinney and J. A. Peterson. 1987. Comparison of microscopic and pinitol techniques in determining legume composition of steer diets. Agron. J. 79: 996-998.

Gedir, J. V. and R. J. Hudson. 2000. Estimating dry matter digestibility and intake in wapiti (Cervus elaphus canadensis) using the double n-alkane ratio technique. Small Ruminant Res. 36: 57-62.

Genstat. 1998. Genstat for Windows, release 4.1. Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire, England.

Gerson, T., A. S. D. King, K. E. Kelly and W. J. Kelly. 1988. Influence of particle size and surface area on in vitro rates of gas production, lipolysis of triacylglycerol and hydrogenation of linoleic acid by sheep rumen digesta or Ruminococcus flaveciens. J. Agric. Sci., Camb. 110: 31-37.

Graham, N. McC. and A. J. Williams. 1962. The effects of pregnancy on the passage of food through the digestive tract of sheep. Aust. J. Agric. Res. 13: 894-900.

Grovum, W. L. and V. J. Williams. 1973. Rate of passage of digesta in sheep. 4. Passage of marker through the alimentary tract and the biological relevance of rate-constants derived from the changes in concentration of marker in faeces. Br. J. Nutr. 30: 313-329.

Hameleers, A. and Mayes, R. W. 1998. The use of n-alkanes to estimate herbage intake and diet composition by dairy cows offered a perennial ryegrass/white clover mixture. Grass and Forage Sci. 53: 164-169.

Hameleers, A and R. W. Mayes 1998b. The use of n-alkanes to estimate supplementary grass silage intake in grazing dairy cows. J. Agric. Sci., Camb. 131: 205-209.

Herrero, M. and N. S. Jessop. 1996. Relationship between in vitro gas production and neutral detergent fibre disappearance in three tropical grasses. Anim. Sci. 62: 682.

Humphreys, L. R. 1991. Tropical pasture utilisation. Cambridge University Press: Cambridge, UK.

Hooper, A. P. and J. G. Welch. 1985. Effects of particle size and forage consumption on functional specific gravity. J. Dairy Sci. 68: 1181-1188.

Illius, A. W. and I. J. Gordon. 1991. Prediction of intake and digestion in ruminants by a model of rumen kinetics integrating animal size and plant characteristics. J. Agric. Sci. 116: 145-157.

Illius, A. W. and I. J. Gordon. 1999. The physiological ecology of mammalian herbivory. *In*: H. G. Jung and G. C. Fahey, Jr., eds. Proceedings of the Vth international symposium on the nutrition of herbivores. San Antonio, Texas, 11-17 April 1999. p. 71-96.

Illius, A. W. and N. S. Jessop. 1996. Metabolic constraints on voluntary intake in ruminants. J. Anim. Sci. 74: 3052-3062.

Jessop, N.S. and M. Herrero. 1996. Failure to account for soluble components results in inaccurate parameter estimation using the in vitro gas production technique. Anim. Sci. 62: 626-627.

Jung, H. G. and M. S. Allen. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci. 73: 2774-2790.

Kaske, M. and W. V. Engelhardt. 1990. The effect of size and density on mean retention time of particles in the gastrointestinal tract of sheep. Br. J. Nutr. 63: 457-465.

Kaske, M., S. Hatiboglu and W. V. Engelhardt. 1992. The influence of density and size of particles on rumination and passage from the reticu-lorumen of sheep.Br. J. Nutr. 67: 235-244

Kennedy, P. M. 1995. Intake and digestion in swamp buffaloes and cattle. 4. Particle size and buoyancy in relation to voluntary intake. J. Agric. Sci., Camb. 124: 277-287.

Kennedy, P. M. and D. P. Poppi. 1984. Critical particle size in sheep and cattle. *In*: P.M. Kennedy, ed. Techniques in Particle Size Analysis of Feed and Digesta in Ruminants. Occasional publication number 1. Edmonton: Canadian Society of Animal Science. p. 170.

Kennedy, P.M. and M. R. Murphy. 1988. The nutritional implications of differential passage of particles through the ruminant alimentary tract. Nutr. Res. Rev. 1:189-208.

Kennedy, P. M. and P. T. Doyle. 1993. Particle-size reduction by ruminants- effects of cell wall composition and structure. *In*: Forage Cell Wall Structure and Digestibility. ASA-CSSA-SSSA, Madison, Wisconsin, USA. p.499-534.

Kennedy, P. M., R. J. Christopherson and L. P. Milligan. 1976. The effect of cold exposure of sheep on digestion, rumen turnover time and efficiency of microbial synthesis. Br. J. Nutr. 36: 231-242.

King, K. W. and W. E. C. Moore. 1957. Density and size as factors affecting passage of ingesta in the bovine and human digestive tracts. J. Dairy Sci. 40: 528-536.

Kotb, A. R. and T. D. Luckey. 1972. Markers in nutrition. Nutr. Abs. Rev. 42: 813-845.

Lascano, C. E. and V. P. B. Euclides. 1996. Nutritional quality and animal production of Brachiaria pastures. *In*: J. W. Miles, B. L. Maass and C. B. do Valle, eds. Brachiaria: Biology, Agronomy and Improvement. CIAT, Cali, Colombia.pp. 106-123.

Lallès, J. P., E. Delval and C. Poncet. 1991. Mean retention time of dietary residues within the gastrointestinal tract of the young ruminant: a comparison of non-compartmental (algebraic) and compartmental (modeling) estimation methods. Anim. Feed. Sci. Technol. 35: 139-159.

Laredo, M. A., G. D. Simpson, D. J. Minson and C. G. Orpin 1991. The potential for using n-alkanes in tropical forages as a marker for the determination of dry matter intake by grazing ruminants. J. Agric. Sci., Camb. 117: 355-361.

Laredo, M. A. and D. J. Minson. 1975. The effect of pelleting on the voluntary intake and digestibility of leaf and stem fractions of three grasses. Br. J. Nutr. 33: 159-170.

Leaver, J. R., R. C. Campling and W. Holmes. 1969. The effect of level of feeding on the digestibility of diets for sheep and cattle. Anim. Prod. 11: 11-18.

Leng, R. A., N. Jessop and J. Kanjanapruthipong. 1993. Control of feed intake and the efficiency of utilisation of feed by ruminants. *In*: D. J. Farrel, ed. Recent Advances in Animal Nutrition in Australia. University of New England, Armidale. p. 70-88.

Lindberg, J. E. 1985. Retention time of chromium-labelled feed particles and of water in the gut of sheep given hay and concentrate at maintenance. Br. J. Nutr. 53: 559-567.

Luginbuhl, J.-M, K. R. Pond and J. C. Burns. 1990. Changes in ruminal and fecal particle weight distribution of steers fed coastal bermudagrass hay at four levels. J. Anim. Sci. 68: 2864-2873.

Mader, T. R., R. G. Teeter and G. W. Horn. 1984. Comparison of forage labelling techniques for conducting passage rate studies. J. Anim. Sci. 58: 208-212.

Malossini, F., S. Bovolenta, E. Piasentier and M. Valentinotti. 1994. Variability of *n*-alkane content in a natural pasture and in faeces of grazing dairy cows. Anim. Feed. Sci. Technol. 50: 113-122.

Mambrini, M. and J. L. Peyraud. 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurement and calculations based on faecal collection. Reprod. Nutr. Dev. 37: 427-442.

Martz, F. A and R. L. Belyea. 1986. Role of particle size and forage quality in digestion and passage by cattle and sheep. J. Dairy Sci. 69: 1996-2008.

Mayes, R. W., C. S. Lamb and P. M. Colgrove. 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. J. Agric. Sci., Camb. 107: 161-170.

Mayes,R. W., C. S. Lamb and P. M. Colgrove. 1988. Digestion and metabolism of dosed even-chain and herbage odd-chain n-alkanes in sheep. Proceedings of the 12th general meeting of the European Grassland Federation. p.159-163.

Mayes, R. W. and H. Dove. 2000. Measurement of dietary nutrient intake in free-ranging mammalian herbivore. Nutr. Res. Rev. 13: 107-138.

Mayes, R. W., N. A. Beresford, C. S. Lamb, C. L. Barnett, B. J. Howard, B.-E. V. Jones, O. Eriksson, K. Hove, Ø. Pedersen and B. W. Staines. 1994. Novel approaches to the estimation of intake and bioavailability of radiocaesium in ruminants grazing forested areas. The Science of the Total Environment. 157: 289-300.

McBride, B. W., L. P. Milligan and B. V. Turner. 1984. Endoscopic observations of digesta transfer from the reticulorumen to the omasum of cattle. Can. J. Anim. Sci. 64 (suppl.): 84-85.

McLeod, M. N. and D. J. Minson. 1988. Breakdown of large particles by simulated digestion and detrition. J. Anim. Sci. 66: 1000-1004.

McLeod, M. N., and B. R. Smith. 1989. Eating and ruminating behaviour in cattle given forages differing in fibre content. Anim. Prod. 48: 503-511.

McMeniman, N. P. 1997. Methods of estimating intake of grazing animals. XXXIV Reuniao Annual da Sociedade Brasileira de Zootecnia. Anais...27 Julio de 1997, Juiz de Fora-MG. 1997 CD-ROM.

Menke, K. H., L. Raab, A. Salewski, H. Steingrass, D. Fritz and W. Schneider. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci., Camb. 93: 217- 222.

Minitab. 1993. Statistical software, release 9. Minitab Inc., Pennsylvania, USA.

Minson, D. J. 1982. Effects of chemical and physical composition of herbage eaten upon intake. *In*: J. B. Hackered. Nutritional Limits to Animal Production from Pastures. Commonwealth Agricultural Bureaux, Farnham Royal, UK. p. 167-182.

Minson, D. J. 1990. Forage in Ruminant Nutrition. Academic Press, New York.

Moore, J. A., K. R. Pond, M. H. Poore and T. G. Goodwin. 1992. Influence of model and marker on digesta kinetic estimates for sheep. J. Anim. Sci. 70: 3528-3540.

Moore, J. E. and L. E. Sollenberger. 1997. Techniques to predict pasture intake. Proceedings of the international symposium on animal production under grazing (Vicosa, Brazil). p. 81-96

Murphy, M. R. and P. M. Kennedy. 1993. Particle dynamics. *In*: J. M. Forbes and J. France, eds. Quantitative Aspects of Ruminant Digestion and Metabolism. CAB International, Oxon. p. 88-105.

Murphy, M. R., P. M. Kennedy and J. G. Welch. 1989. Passage and rumination of inert particles varying in size and specific gravity as determined from analysis of faecal appearance using multicompartment models. Br. J. Nutr. 62: 481-492.

Neel, J. P. S., E. C. Prigge and E. C. Townsend. 1995. Influence of moisture content of forage on ruminal functional specific gravity and passage of digesta. J. Anim. Sci. 73: 3094-3102.

Newman, J. A., W. A. Thompson, P. D. Penning and R. W. Mayes. 1995. Least-squares estimation of diet composition from n-alkanes in herbage and faeces using matrix mathematics. Austr. J. Agric. Res. 46: 793-805

Newman, J. A. F, Cribari-Neto and M.J. Jensen. 1998. The sensitivity of n-alkane analysis to measurement error: implications for use in the study of diet composition. J. Agric. Sci., Camb. 131: 465-476.

Okine, E. K. and G. W. Mathison. 1991.Reticular contraction attributes and passage of digesta from the ruminoreticulum in cattle fed roughage diets. J. Anim. Sci. 69:2177-2186.

Okine, E. K. and G. W. Mathison. 1991a. Effects of feed intake on particle distribution, passage of digesta, and extent of digestion in the gastrointestinal tract of cattle. J. Anim. Sci. 69:3435-3445.

Pasha, T. N., E. C. Prigge, R. W. Russell and W. B. Bryan. 1994. Influence of moisture content of forage diets on intake and digestion by sheep. J. Anim. Sci. 72: 2455-2463.

Pell, A. and P. Schoefield. 1993. Computerized monitoring of gas production to measure forage digestion in vitro. J. Dairy Sci. 76: 1063-1073.

Poncet, C. 1991. The outflow of particles from the reticulo-rumen. *In*: J. P. Jouany, ed. Rumen microbial metabolism and ruminant digestion. INRA Editions, Paris. p. 297-322.

Pond, K. R., J. H. Matis, H. M. Ferreiro and J. D. Sutton. 1988. Compartment models for estimating attributes of digesta flow in cattle. Br. J. Nutr. 60: 571-595.

Pond, K. R., L. Goode, E. S. Leonard and D. L. Mann. 1984. Intake, digesta fill and flow kinetics pre- and postpartum. Can. J. Anim. Sci. 64 (suppl.): 68-69.

Poppi, D. P., B. W. Norton, D. J. Minson and R. E. Hendricksen. 1980. The validity of the critical size theory for particles leaving the rumen. J. Agric. Sci., Camb. 94: 275-280.

Poppi, D. P., D. J. Minson and J. H. Ternouth. 1981. Studies of cattle and sheep eating leaf and stem fractions of grasses. IV. The retention time in the rumen of large feed particles. Austr. J. Agric. Res. 32: 123-137.

Preston, T. R and R. A Leng. 1987. Matching ruminant production systems with available resources in the tropics and sub-tropics. Penambul Books: Armidale, Australia.

Quiroz, R. A., K. R. Pond, E. A. Tolley and W. L. Johnson. 1988. Selection among nonlinear models for rate of passage studies in ruminants. J. Anim. Sci. 66: 2977-2986.

Rao, P. V. 1998. Statistical research methods in the life sciences. Brooks/Cole Publishing Company: Pacific Grove, California.

Reid, C. S. W. 1986. Digestive physiology: The challenges today and tomorrow. *In*: L. P. Milligan et al., eds. Control of digestion and metabolism in ruminants. Reston, Engelwood Cliffs, New Jersey. p. 540-557.

Romney, D. L., Cadario, F. C., Owen, E. and Murray, A. H. 1997. Comparison of parameters from the Theodorou gas production technique using nitrogen-free and nitrogen-rich media as predictors of DM intake and digestibility. *In*: E. R. Deaville, E. Owen, A. T. Adesogan, C. Rymer, J. A. Huntingdon and T. L. J. Lawrence, eds. In vitro techniques for measuring nutrient supply to ruminants British Society of Animal Science occasional publication no. 22. p. 172 - 174.

Salt, C. A., R. W. Mayes, P. M. Colgrove and C. S. Lamb. 1994. The effects of season and diet composition on the radiocaesium intake by sheep grazing on heather moorland. J. Appl. Ecol. 31: 125-136

Shriver, B. J., W. H. Hoover, J. P. Sargent, R. J. Crawford, Jr. and W. V. Thayne. 1986. Fermentation of a high concentrate diet as affected by ruminal pH and digesta flow. J. Dairy Sci. 69: 413-419.

Smith, L. W. B. T. Weinland, D. R. Waldo and E. C. Leffel. 1983. Rate of cell wall particle size reduction in the rumen. J. Dairy Sci. 66: 2124-2136.

Stakelum, G. and P. Dillon. 1990. Dosed and herbage alkanes as feed intake predictors with dairy cows: the effect of feeding level and frequency of perennial ryegrass. Proceedings of the VII European Grazing Workshop (Wageningen, The Netherlands) October 1990.

Sutherland, T. M. 1988. Particle separation in the forestomachs of sheep. *In*: A. Dobson and M. J. Dobson, eds. Aspects of Digestive Physiology in Ruminants. Cornell University Press, Ithaca, New York. p. 43-73.

Tetlow, R. M. and R. J. Wilkins. 1974. The effects of method of processing on the intake and digestibility by lambs of dried perennial rye-grass and tall fescue. Anim. Prod. 19: 193-200.

Thielemans, R. F., E. Francois, C. Bodart and A. Thewis. 1978. Mesure du transit gastro-intestinal chez le porc à l'aide radiolanthenides comparaison avec le mouton. Ann. Biol. Anim., Biochim. Biophys. 18: 237-247.

Troelson, J. E. and J. B. Campbell. 1968. Voluntary consumption of forage by sheep and its relation to the size and shape of particles in the digestive tract. Anim. Prod. 10: 289-296.

Uden, P., P. E. Colucci and P. J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food. Agric. 31: 625-632.

Unal, Y. and P. C. Garnsworthy. 1999. An alternative method of administering alkane markers for estimating intake in dairy cows. Proceedings of British Society of Animal Science. p. 91 (abstract).

Van Soest, P. J. 1982. Nutritional Ecology of the Ruminant. O&B Books, Corvallis, Oregon.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press, Ithaca, New York.

Van Soest, P. J., C. J. Sniffen and M. S. Allen. 1988. Rumen dynamics *In*: Dobson, A. and M. J. Dobson, eds. Aspects of Digestive Physiology in Ruminants. Cornell University Press, Ithaca, New York. p. 21-42.

Varga, G. A. and W. H. Hoover. 1983. Rate and extent of neutral detergent fiber degradation of feedstuffs *in situ*. J. Dairy Sci. 66: 2109-2115.

Vulich, S. A., E. G. O'Riordan and J. P. Hanrahan. 1991. Use of n-alkanes for the estimation of herbage intake in sheep: accuracy and precision of the estimates. J. Agric. Sci., Camb. 116: 319-323.

Waghorn, G. C., C. S. W. Reid, M. J. Ulyatt and A. John. 1986. Feed comminution, particle composition and distribution between the four compartments of the stomach in sheep fed chopped lucerne hay at two feeding frequencies and intake levels. J. Agric. Sci. 106: 287-296.

Warren, W. P., F. A. Martz, K. H. Asay, E. S. Hilderbrand, C. G. Payne and J. R. Vogt. 1974. Digestibility and rate of passage by steers fed tall fescue, alfalfa and orchardgrass hay in 18 and 32 C ambient temperatures. J. Anim. Sci. 39: 93-96.

Welch, J. G. 1967. Appetite control in sheep by indigestible fibers. J. Anim. Sci. 26: 849-854.

Welch, J. G. 1982. Rumination, particle size and passage from the rumen. J. Anim. Sci. 54: 885-894.

Welch, J. G. 1990. Inert plastics as indicators of physiological processes in the gastrointestinal tract of ruminants. J. Anim. Sci. 68: 2930-2935.

Welch, J. G. and A. M. Smith. 1978. Particle sizes passed from the rumen. J. Anim. Sci. 46: 309-312.

Weston, R. H. 1985. The regulation of feed intake in herbage-fed ruminants. Proceedings of the Nutrition Society of Australia 10: 55-62.

Weston, R. H. 1988. Factors limiting the intake of feed by sheep. XI. The effect of pregnancy and early lactation on the digestion of a medium-quality roughage. Aust. J. Agric. Res. 39: 659-669.

Weston, R. H. 1996. Some aspects of constraint to forage consumption by ruminants. Aust. J. Agric. Res. 47: 175-197.

Weston, R. H. and J. P. Hogan. 1967. Digestion of chopped and ground roughages by sheep. 1. The movement of digesta through the stomach. Aust. J. Agric. Res. 18: 789-801.

Weston, R. H. and P. M. Kennedy. 1984. Various aspects of reticulorumen digestive function in relation to diet and digesta particle size. *In*: P. M. Kennedy, ed. Techniques in Particle Size Analysis of Feed and Digesta in Ruminants. Occasional publication number 1. Canadian Society of Animal Science. Edmonton. p. 1-17.

Wilson, J. R. 1993. Organization of forage plant tissues. *In*: H. G. Jung, ed. Forage Cell Wall Structure and Digestibility. ASA-CSSA-SSSA, Madison, Wisconsin, USA. p.1-31.

Wilson, J. R. and P. J. Kennedy. 1996. Plant and animal constraints to voluntary feed intake associated with fibre characteristics and particle breakdown and passage in ruminants. Aust. J. Agric. Res. 47: 199-225.

Wilson, J. R., M. N. McLeod and D. J. Minson. 1989. Particle size reduction of the leaves of a tropical and a temperate grass by cattle. 1. Effect of chewing during eating and varying times of digestion. Grass and Forage Sci. 44: 55-63.

Worrell, M. A., D. C. Clanton, W. W. Stroup and J. T. Nichols. 1986. The effect of harvest date on meadow hay quality. II. Particle size degradation and particulate passage from the rumen of growing cattle. J. Anim. Sci. 63: 1538-1546.

Wylie, M. J., W. C. Ellis, J. M. Matis, E. M. Bailey, W. D. James and D. E. Beever. 2000. The flow of forage particles and solutes through segments of the digestive tracts of cattle. Br. J. Nutr. 83: 295-306.

Young, B. A. 1983. Ruminant cold stress; effect of production. J. Anim. Sci. 57: 1601-1607.

Faecal concentrations (mg of alkane/g of faecal DM) of each alkane used to mark particle pools dosed to the bulls. The listed data are for each bull used in the experiment and bulls were grouped into 5 groups of 4 bulls according to weight. Blank spaces indicate that no sample could be obtained at sampling time. Hours indicate sampling times.

Group I

116-7 (weight 85kg)

Hours	c32 (SD)	c30 (LD)	c28 (SB)	c26 (LB)
0	0.007084	0.002276	0	0
8	0	0	0.004614	0.004372
12				
19	0.01555	0.007846	0.015185	0.007799
24	0.2066	0.120914	0.187103	0.063462
30	0.347271	0.212923	0.293381	0.129068
36	0.386818	0.252962	0.328399	0.176248
44	0.29005	0.211504	0.226177	0.126133
48	0.253957	0.20412	0.190656	0.136772
54	0.211374	0.202202	0.149123	0.121514
60	0.166444	0.165112	0.114197	0.100837
68	0.167657	0.170571	0.10613	0.102156
72	0.108037	0.113452	0.072665	0.066507
79	0.093537	0.114105	0.059884	0.062735
84	0.079406	0.08869	0.047342	0.053657
92	0.055022	0.074017	0.035114	0.038912
96	0.058732	0.057759	0.032605	0.037501
103	0.045665	0.049275	0.025118	0.032112
108	0.03503	0.04706	0.021207	0.026269
116	0.031598	0.035763	0.017566	0.021817
127	0.025304	0.025039	0.012959	0.017109
140	0.014725	0.012467	0.009349	0.009185

118-7 (weight 95kg)

Hours	c32	c30	c28	c26
0	0	0	0	0
9	0.003273	0.000821	0.00661	0.00374
13	0.044944	0.041867	0.043528	0.007799
20	0.189207	0.095597	0.177548	0.059894
25	0.268128	0.153321	0.244626	0.122863
31	0.241591	0.175261	0.204159	0.138391
37				
45	0.179963	0.153529	0.134235	0.116944
49	0.174019	0.154558	0.129026	0.133453
55	0.135966	0.159358	0.103004	0.113873
61				
69	0.085539	0.094483	0.062415	0.066594
73	0.078597	0.087303	0.058489	0.05813

80	0.049313	0.055199	0.03144	0.036204
85				
93	0.038311	0.043503	0.021896	0.031738
97	0.041159	0.045847	0.019265	0.024979
104	0.031295	0.032721	0.015213	0.0157
109				
116				
128	0.017098	0.017313	0.009059	0.006881
141	0.007783	0.009759	0.00634	0.004622

136-7 (weight 90kg)

Hours	c32	c30	c28	c26
0	0	0	0	0
8.5	0.00047	0.000755	0.000367	0.0003
13	0.065983	0.070721	0.059142	0.044885
20	0.450177	0.465739	0.40354	0.240872
25	0.475058	0.495619	0.406056	0.27618
31	0.40011	0.436783	0.290783	0.214948
37	0.341626	0.386344	0.252155	0.204685
45	0.172651	0.213412	0.123043	0.114848
49	0.139284	0.183676	0.090511	0.108917
55	0.091241	0.129879	0.058983	0.071061
61	0.080763	0.111212	0.049733	0.059183
69	0.058167	0.08199	0.032819	0.051025
73	0.047789	0.068242	0.027395	0.037742
80	0.034462	0.046559	0.01697	0.023839
85	0.026621	0.035321	0.01284	0.015777
93	0.016944	0.023864	0.009088	0.012473
97	0.015373	0.021015	0.007112	0.010814
103				
109	0.008294	0.011046	0.004893	0.005447
117	0.006872	0.008569	0.004407	0.005328
127	0.007268	0.007179	0.00224	0.002014
141	0.004892	0.004325	0.002385	0.000915

94-7 (weight 80kg)

Hours	c32	c30	c28	c26
0	0	0	0	0
9	0.000683	0.000674	0.010655	0.00024
13	0.022782	0.023567	0.059347	0.005174
20	0.201073	0.258308	0.265074	0.105208
25	0.280435	0.332018	0.302195	0.146943
31	0.228566	0.287673	0.229403	0.148813
37	0.22323	0.300335	0.205599	0.164463
45	0.17659	0.236324	0.14326	0.133262
49	0.146853	0.204907	0.11825	0.12038
55	0.105406	0.154817	0.08768	0.090871
61	0.106427	0.15816	0.081794	0.081843
69	0.097964	0.146716	0.067893	0.083978

73	0.071333	0.114809	0.055956	0.070047
80	0.062703	0.092532	0.043516	0.055712
85	0.052957	0.076546	0.034794	0.046108
93	0.035471	0.058112	0.027893	0.029527
97	0	0.002124	0.008816	0.004395
104	0.024339	0.035839	0.018938	0.020071
109	0.021533	0.031821	0.017411	0.017167
117	0.014485	0.023462	0.014715	0.014374
128	0.003996	0.013239	0.010427	0.007269
141	0.006395	0.011874	0.009694	0.006863

Group II

72-7 (weight 140kg)

Hours	c32	c30	c28	c26
0	0	0	0	0
8	0	0	0	0
12.5	0.016653	0.016432	0.026687	0.01622
19.5	0.170052	0.139252	0.182652	0.10934
24.5	0.16672	0.132987	0.162222	0.09439
30.5	0.121059	0.118026	0.103439	0.088593
36.5	0.10327	0.105541	0.083629	0.081888
44.5	0.072494	0.086791	0.05756	0.062138
48.5	0.059047	0.070941	0.038857	0.0478
54.5	0.048827	0.062287	0.03467	0.045206
60.5	0.033153	0.042887	0.020005	0.029992
68.5	0.012951	0.020337	0.010281	0.015955
72.5	0	0.007484	0.004457	0.008633
79.5	0.001645	0.007947	0.003869	0.006241
84.5	0.004127	0.006116	0.002833	0.003928
92.5	0	0.000718	0.001918	0.002861
96.5	0	0.001109	0.001197	0.003982
103.5	0	0	0.000624	0.000768
108.5	0	0.001425	0.001426	0.000877
116.5	0	0	0.001107	0.000357
127.5	0	0	0	0
140.5	0	0.000133	0.000327	0

74-7 (weight 155kg)

Hours	c32	c30	c28	c26
0	0	0	0	0
7.5	0.002514	0.010124	0.008068	0.005542
12	0.056746	0.040273	0.083781	0.034123
19	0.07244	0.050923	0.076716	0.067063
24	0.102295	0.12127	0.0929	0.061796
30	0.100591	0.10621	0.090268	0.070442
36	0.093258	0.113383	0.083835	0.077271
44	0.062146	0.079489	0.053585	0.054838
48	0.042532	0.059217	0.039707	0.040006

54	0.044531	0.062139	0.033441	0.03618
60	0.041411	0.051842	0.028305	0.038072
68	0.027799	0.041533	0.01909	0.021727
72	0.025057	0.047318	0.015274	0.01835
79	0.014852	0.026226	0.011755	0.013882
84	0.017613	0.024082	0.011821	0.012802
92	0.011959	0.020099	0.009796	0.012026
96	0.053338	0.067961	0.025189	0.032946
103	0.008945	0.015836	0.006507	0.008464
108	0.010203	0.017819	0.007772	0.007058
116	0.009106	0.01765	0	0.006516
127	0.002875	0.01076	0	0.004612
140	0.009884	0.014825	0	0.004895

90-7 (weight 140kg)

Hours	c32	c30	c28	c26
0	0.001448	0.000992	0	7.35E-05
8	0	0	0	0
12.5	0.020522	0.007084	0.016838	0.002654
19.5	0.077136	0.031824	0.073161	0.02237
24.5	0.173064	0.086528	0.16609	0.059408
30.5	0.178237	0.14643	0.158723	0.117725
36.5	0.155358	0.134336	0.130038	0.100879
44.5	0.101109	0.107333	0.088357	0.080672
48.5	0.063028	0.088327	0.063536	0.065657
54.5	0.069078	0.091457	0.05395	0.055111
60.5				
68.5	0.051041	0.067276	0.037163	0.040054
72.5	0.041418	0.06051	0.032347	0.033318
79.5	0.019804	0.036737	0.021099	0.024314
84.5	0.029183	0.040268	0.021455	0.021342
92.5	0.019663	0.029497	0.015847	0.017582
96.5	0.014709	0.02365	0.013909	0.012775
102.5	0.011208	0.023024	0.012494	0.01203
108.5	0.011989	0.016741	0.011169	0.007837
116.5	0.011229	0.015146	0.010303	0.007325
127.5	0.006844	0.026077	0.008493	0.004029
140.5	0.00459	0.005702	0.006761	0.002456

92-7 (weight 150kg)

Hours	c32	c30	c28	c26
0	0.0035	0	0	0
9	0	0.004229	0.005152	8.32E-05
13	0.08778	0.049247	0.113924	0.023118
20	0.118805	0.088811	0.100798	0.050039
25	0.122929	0.100211	0.096598	0.053756
31	0.10742	0.107807	0.07052	0.064377
37	0.088007	0.124971	0.075529	0.094386
45	0.052418	0.070505	0.035354	0.046993

49	0.040924	0.044876	0.026965	0.031983
55	0.069545	0.088914	0.049241	0.055418
61	0.034438	0.057565	0.022588	0.036604
69	0.010429	0.026291	0.008959	0.017987
73	0.009139	0.019961	0.008292	0.016018
80	0.006621	0.016022	0.005933	0.013277
85	0.014997	0.022008	0.007347	0.013899
93	0.00687	0.015823	0.004261	0.011515
97	0.005452	0.011813	0.00459	0.008081
104	0.002651	0.00829	0.002883	0.007202
109	0.006271	0.009484	0.002992	0.005884
117	0.003669	0.004567	0.00216	0.005765
128	0.005977	0.005844	0.003758	0.004136
141	0.003014	0.002847	0.000974	0.002066

Group III

849 (weight 300kg)

Hours	c32	c30	c28	c26
0	0	0	0	0
6	0.004151	0.002034	0.004687	0.002464
11.5	0.016581	0.006042	0.013411	0.008072
18.5	0.093832	0.056218	0.090795	0.04547
23.5	0.152788	0.125597	0.161663	0.098911
29.5	0.124465	0.107244	0.134117	0.084331
35.5	0.103142	0.120835	0.103818	0.102068
41.5	0.071354	0.097875	0.07054	0.095475
47.5	0.073649	0.085152	0.063798	0.072262
53.5	0.046738	0.064999	0.043472	0.057978
59.5	0.043349	0.055934	0.03715	0.049198
66.5	0.027717	0.040297	0.025592	0.037983
71.5	0.022123	0.033859	0.021521	0.032335
77.5	0.022858	0.031122	0.018896	0.026083
83.5	0.01182	0.019498	0.01234	0.017962
90.5	0.008897	0.014252	0.00961	0.015174
95.5	0.008934	0.014986	0.013295	0.017069
101.5	0.008663	0.013875	0.012382	0.013218
107.5	0.003678	0.00891	0.009976	0.012784
115.5	0.002163	0.00936	0.008074	0.010571
126.5	0.010088	0.009311	0.008451	0.009462
138.5	0.002206	0.005187	0	0.00852

850 (weight 325kg)

Hours	c32	c30	c28	c26
0	0	0.000335	0	0
6	7.25E-05	0	5.29E-05	0.000263
11.5	0.000424	0.00077	0.003874	0.001891
18.5	0.059399	0.04704	0.068413	0.030513
23.5	0.134784	0.09459	0.13203	0.070859
29.5	0.126392	0.113971	0.121502	0.101776

35.5	0.093628	0.100965	0.082825	0.07579
41.5	0.068079	0.084003	0.064861	0.060284
47.5	0.051123	0.062571	0.044359	0.043849
53.5	0.053888	0.060946	0.040436	0.042113
59.5	0.032714	0.05773	0.027409	0.032977
66.5	0.028069	0.040971	0.022371	0.028511
71.5	0.02025	0.034144	0.016354	0.023074
77.5	0.010081	0.020953	0.011128	0.016537
83.5	0.009936	0.017424	0.00812	0.010902
90.5	0.006676	0.014224	0.006537	0.012813
95.5	0.009639	0.015278	0.006164	0.00912
101.5	0.006356	0.01148	0.004528	0.006152
107.5	0.004326	0.009923	0.004254	0.005656
115.5	0	0.005918	0.001599	0.004134
126.5	0.006845	0.008206	0.00245	0.002143
138.5	0	0.003072	0.000651	0.001607

844 (weight 335kg)

Hours	c32	c30	c28	c26
0	0.000416	0	0	0
6	0	0.000905	0	0
11.5	0	0.00052	0.014227	0.006184
18.5	0.037359	0.022094	0.057046	0.025639
23.5	0.123431	0.085502	0.150291	0.074723
29.5	0.182492	0.141545	0.171073	0.118268
35.5	0.127483	0.129483	0.129804	0.104334
41.5	0.110904	0.127311	0.102105	0.087953
47.5	0.087585	0.101675	0.088529	0.076305
53.5	0.056327	0.078187	0.061246	0.059623
59.5				
66.5	0.035382	0.053337	0.036914	0.040892
71.5	0.02236	0.041275	0.028685	0.030121
77.5	0.013199	0.02494	0.021121	0.023294
83.5	0	0.010031	0.017497	0.015648
90.5	0	0.004939	0.014632	0.014231
95.5	0.002121	0.009677	0.013073	0.011882
101.5	0.00677	0.011616	0.014156	0.01735
107.5	0.010341	0.011547	0.013801	0.011024
115.5	0	0.004173	0.011915	0.008144
126.5	0	0.001356	0	0.006476
138.5	0	0.000266	0	0.006252

859 (weight 280kg)

Hours	c32	c30	c28	c26
0	0.002576	0.002056	0.001463	0.000976
6	0	0	0	0
11	0.012123	0.005878	0.008926	0.006474
18	0.11543	0.067901	0.097908	0.062827
23	0.1541	0.111364	0.137367	0.090408

29	0.131585	0.120077	0.114264	0.074842
35	0.106015	0.113325	0.087215	0.077789
41	0.084733	0.095555	0.067292	0.06449
47	0.085178	0.092972	0.06407	0.072466
53	0.039307	0.05439	0.032453	0.032266
59	0.049942	0.065088	0.036533	0.040228
66	0.059026	0.065039	0.024014	0.024075
71	0.030497	0.042453	0.020138	0.022886
77	0.017747	0.031198	0.01105	0.016196
83	0.012434	0.020719	0.007794	0.010546
90	0.007874	0.016097	0.005903	0.007683
95	0.005744	0.012035	0.003578	0.005646
101	0.009173	0.013707	0.003396	0.005165
107	0.002827	0.009468	0.001788	0.003318
115	0	0.001325	0	0
126	0.000859	0.011904	0.000568	0.001044
138	0	0.000846	0	0

Group IV

788 (weight 445kg)

Hours	c32	c30	c28	c26
0	0.003562	0.002256	0.001678	0.000989
6	0	0	0	0
11.5	0.006084	0.001659	0.00505	0.002044
18.5	0.042806	0.031621	0.033903	0.014283
23.5	0.097044	0.052465	0.08993	0.042316
29.5	0.106428	0.087272	0.100099	0.077171
35.5	0.083224	0.079309	0.070427	0.052578
41.5	0.068419	0.069286	0.053978	0.045623
47.5	0.049431	0.058139	0.034327	0.031857
53.5	0.031492	0.042234	0.019597	0.020503
59.5	0.018269	0.025818	0.008535	0.020339
66.5	0.020177	0.025923	0.007556	0.009842
71.5	0.013902	0.017048	0.003657	0.006626
77.5	0.011976	0.013793	0.000686	0.003466
83.5	0.005558	0.007119	0	0
90.5	0.002566	0.004814	0	0
95.5	0.004229	0.004426	0	0
101.5	0	0	0	0
107.5	0.001083	0.000597	0	0
115.5	0.003652	0.001671	0	0
126.5	0.000307	0	0	0
138.5	0	0	0	0

810 (weight 455kg)

Hours	c32	c30	c28	c26
0	0	0	0	0
6.5	0.001553	0.000944	0	0
12	0.04256	0.015887	0.041703	0.018565

19	0.109458	0.059944	0.128882	0.056526
24	0.128274	0.085686	0.129005	0.077765
30	0.095701	0.071269	0.101725	0.060047
36	0.101372	0.08649	0.098151	0.070236
42	0.064572	0.062086	0.062738	0.050598
48	0.060115	0.086945	0.058784	0.052418
54	0.042943	0.056819	0.049263	0.053113
60	0.021618	0.043329	0.034438	0.036239
67	0.021543	0.041427	0.029161	0.040513
72	0.017406	0.043428	0.026627	0.028666
78	0.024904	0.032992	0.026886	0.025684
84	0.017648	0.028231	0.021583	0.023368
91	0.011872	0.019086	0.017115	0.018423
96	0.013807	0.016309	0.017639	0.013722
102	0.015897	0.019683	0.01685	0.01632
108	0.004562	0.010429	0.01306	0.015186
116	0.005771	0.010949	0.012637	0.011235
127	0.001973	0.012291	0	0.008339
139	0	0.010381	0	0

796 (weight 470kg)

Hours	c32	c30	c28	c26
0	0.000754	0.00079	3.66E-05	0
5.5	0	0	0	0
11	0.008988	0.002026	0.009657	0
18	0.017574	0.009834	0.015386	0
23	0.093247	0.050938	0.088398	0.054518
29	0.102546	0.077249	0.088634	0.053043
35	0.092123	0.087669	0.081501	0.095095
41	0.066696	0.079084	0.056467	0.059417
47	0.074069	0.073511	0.056807	0.055864
53	0.043719	0.054196	0.037158	0.047684
59	0.031728	0.050136	0.02861	0.033258
66	0.009575	0.023176	0.016161	0.021979
71	0.030154	0.04422	0.021832	0.027182
77	0.000294	0.014989	0.009901	0.014319
83	0.015967	0.028837	0.014342	0.020349
90	0.004913	0.019442	0.006471	0.011843
95	0.011303	0.021083	0.009711	0.015125
101	0.005521	0.013533	0.006043	0.009956
107	0	0.00699	0.003654	0.00709
115	0.003888	0.00924	0.004197	0.007785
126	0	0.005742	0.002094	0.005358
138	0	0.002522	0.001087	0.003743

832 (weight 415kg)

Hours	c32	c30	c28	c26
0	0.000664	0.000459	0	0
6.5	0	0	0	0

12	0.002043	0.001964	0.012382	0
19	0.039742	0.028491	0.054133	0.02905
24	0.096416	0.056885	0.108038	0.064838
30	0.116693	0.071915	0.119883	0.073893
36	0.121245	0.079795	0.103212	0.076229
42	0.091586	0.071856	0.087131	0.060778
48	0.076586	0.070508	0.072394	0.070873
54	0.048352	0.062101	0.051307	0.044465
60	0.058717	0.057448	0.049719	0.040648
67	0.034429	0.04809	0.035102	0.031125
72	0.0357	0.038562	0.030921	0.030605
78	0.026423	0.03373	0.026182	0.024362
84	0.021225	0.028896	0.024063	0.021638
91	0.002747	0.011251	0.015868	0.013354
96	0.010857	0.015595	0.016523	0.015038
102	0.011581	0.017151	0.016564	0.014615
108	0.006782	0.012073	0.013001	0.017836
116	0.001427	0.007497	0.011482	0.008782
127	0	0.000989	0.009517	0.006986
139	0	0.002502	0.009097	0

Group V

25 (weight 416kg)

Hours	c32	c30	c28	c26
0	0	0	0	0.000122
7	0.000446	0.000122	0.000149	0
12	0.011941	0.006381	0.009537	0.004938
19	0.021341	0.011417	0.033669	0.01408
24	0.082496	0.056213	0.089608	0.052657
30	0.088928	0.080814	0.091602	0.073488
36	0.089531	0.089404	0.089868	0.073452
42	0.087567	0.076403	0.076884	0.06334
48	0.062519	0.067233	0.058729	0.046733
54	0.056182	0.055647	0.046507	0.043781
60	0.047099	0.054473	0.035898	0.034505
67	0.044685	0.050663	0.031702	0.030171
72	0.034837	0.041367	0.024504	0.02758
78	0.025228	0.032752	0.019047	0.02118
84	0.022229	0.024166	0.014195	0.015426
91	0.018363	0.024083	0.011297	0.01439
96	0.018451	0.022902	0.010786	0.011265
102	0.010307	0.018622	0.007118	0.010595
108	0.009806	0.013681	0.006356	0.012212
116	0.008036	0.011707	0.005107	0.006559
127	0.004137	0.005897	0.002531	0.003409
139	0.00295	0.004296	0.002498	0.004032

M2-6 (weight 403kg)

Hours	c32	c30	c28	c26

0				
6	0	0	0	0
11	0.005174	0.002576	0.006364	0
18	0.038961	0.023806	0.043114	0.021309
23	0.110822	0.069539	0.114765	0.061538
29	0.105907	0.086802	0.111735	0.06709
35	0.090619	0.085466	0.087924	0.066645
41	0.076193	0.076365	0.06938	0.075282
47	0.068904	0.069824	0.057007	0.06086
53	0.038231	0.047974	0.038426	0.038159
59	0.032436	0.045467	0.032365	0.039148
66	0.021299	0.036596	0.023525	0.026589
71	0.026338	0.036533	0.024548	0.029703
77	0.024739	0.036635	0.020405	0.026442
83	0.000726	0.011475	0.009832	0.015719
90	0.01513	0.022089	0.01385	0.015778
95	0.008273	0.016715	0.00997	0.013781
101	0.005429	0.012466	0.008109	0.013153
107	0.006357	0.012475	0.00874	0.011023
115	0.001735	0.009986	0.007167	0.008991
126	0.003136	0.008274	0.006143	0.007715
138	0.000331	0.006365	0.005069	0.005799

M20-5 (weight 462kg)

Hours	c32	c30	c28	c26
0	0	0	0	0
6	0.002902	0.001127	0.00113	0.000368
12	0.042709	0.014338	0.030807	0.023044
19	0.119035	0.07564	0.110827	0.046873
24	0.139589	0.092472	0.127326	0.069108
30	0.115907	0.092534	0.105275	0.065817
36	0.088088	0.082767	0.074255	0.05923
42	0.078597	0.083322	0.071568	0.061953
48	0.070042	0.080642	0.054883	0.051412
54	0.048193	0.058986	0.036261	0.036147
60	0.037939	0.054376	0.030423	0.027034
67	0.032387	0.047246	0.023289	0.025614
72				
78	0.014993	0.03525	0.010986	0.013928
84	0.020404	0.027566	0.010066	0.00974
91	0.010436	0.021079	0.00493	0.005465
96	0.00881	0.013374	0.00497	0.004575
102	0.01244	0.016858	0.004222	0.004269
108				
116	0.002254	0.009197	0.001525	0.002239
127	0.005872	0.008149	0.002518	0
139	0.001272	0.00408	0.000154	0

M18-5 (weight 472kg)

Hours	c32	c30	c28	c26
0	0.000425	0.000153	0	0
6	0	0	4.77E-05	5.22E-05
11	0.004742	0.001987	0.001386	0.000536
18	0.033889	0.013105	0.037735	0.01736
23	0.054485	0.038078	0.054706	0.021028
29				
35	0.093731	0.095914	0.085469	0.0641
41	0.081468	0.086018	0.071195	0.058302
47	0.064406	0.076534	0.055626	0.049401
53	0.050025	0.059519	0.041112	0.044084
59	0.019516	0.033541	0.022707	0.023753
66	0.025808	0.038189	0.02235	0.024222
71	0.023328	0.035831	0.019389	0.022218
77	0.019036	0.029599	0.016308	0.020887
83	0.015934	0.024743	0.010792	0.014092
90	0.009348	0.015749	0.007236	0.008496
95	0.008359	0.018817	0.015554	0.017036
101	0.006643	0.012554	0.012722	0.014473
107	0.003256	0.014405	0.011207	0.012396
115	0.0033	0.010604	0.011692	0.012508
126	0.001402	0.00736	0.010056	0.0105
138	0.004418	0.005108	0.003518	0.004846